Novel biomimetic polymersomes as polymer therapeutics for drug delivery

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Received 9 October 2004; accepted 14 June 2005
Available online 9 September 2005

Abstract

Novel amphiphilic diblock copolymers, cholesterol-end-capped poly(2-methacryloyloxyethyl phosphorylcholine) (CMPC), which have poly(2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)) as hydrophilic segment and cholesterol as hydrophobic segment, was specially designed as drug delivery systems. Fluorescence probe technique and transmission electron microscope (TEM) characterizations indicated that this novel amphiphilic copolymer formed micelles structure in water and the critical micelle concentration (CMC) was determined to be $1.57 \times 10^{-7}$ mol/l. A commercial obtained polymeric amphiphiles, Cholesterol end capped PEO (CPEO), which had a similar structure with CMPC, was used as a control in the cytotoxicity test. While CPEO showed obvious cytotoxicity, cytotoxicity of this novel amphiphiles was not observed as indicated by cell culture. Anti-cancer drug adriamycin (ADR) was incorporated into the micelles by oil-in-water method. The size of the drug-containing micelles was less than 200 nm, and the size distribution of the drug-containing micelles showed a narrow and monodisperse unimodal pattern. The release rate of ADR from the nanosphere was slow and the release continued over 7 days and the release rate decreased with the increase of molecular weights of the copolymer and the amount of the drug entrapped. These experimental results suggested that the nanoparticles prepared from CMPC block copolymers could be a good candidate for injectable drug delivery carrier.

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Keywords: 2-Methacryloxyethyl phosphorylcholine; Amphiphile; Drug delivery; Cholesterol; Biomimetic

1. Introduction

Polymeric micelles based on amphiphilic polymers has gained an increasing interest as a carrier system of drugs and found vast applications in drug delivery [1–3]. These micelles feature a lipophilic core that solubilizes hydrophobic drugs and a hydrophilic shell that make the entire assembly water-soluble. The ability of micelles to solubilize hydrophobic drugs expands the pharmaceutical potential of lipophilic drug molecules. Small dimensions of polymeric micelles allow for extravasation at tumor sites due to higher permeability...
of tumor blood vessels, leading to accumulation of micelle-encapsulated drugs within tumors [4]. Furthermore, small particles can escape renal excretion, as well as avoiding components of the reticular endothelial system (RES). RES recognition is considerably lowered for particles less than ca. 200 nm and with appropriately modified surfaces [5–12].

Amphiphilic diblock copolymers composed of a hydrophilic and a hydrophobic segment can form micellar structures with either a hydrophobic compact inner core and a hydrophilic swollen outer shell or vice versa, depending on the solvent, which thermodynamically favors either of the two blocks [13,14]. Drugs to be delivered can be covalently attached prior to micellization or vesicle formation or entrapped into the micellar core or polymersome by the use of various techniques like solution/precipitation, salting out process, or solvent evaporation method [15–17]. Towards this end, amphiphilic diblock copolymers composed of hydrophilic chain, in particular polyethylene glycol (PEG), PEG-based hydrogels, polyacrylamide, dextran, polyvinyl alcohol, polyvinylpyrrolidone, etc. have been widely studied [18–20]. Among these materials, PEG is a commonly used material. However, it was found that PEG with lower molecular weight (i.e. less than 3000) was cytotoxic in the body [21,22].

Liposomes have advantages over other delivery systems. Liposome can be used as a non-toxic, biodegradable and non-immunogenic system to solubilize drugs that have low aqueous solubility [23,24]. Moreover, the unique properties of liposomes, which include a large aqueous interior and a biocompatible lipid exterior, make them into ideal candidates for drug delivery [25–27]. This has led to intensive investigations of liposomes to be studied as sustained drug delivery systems [28]. However, the biological instability and rapid uptake of liposomes by the reticuloendothelial system (RES) after injection has hinders their exploitation in the delivery of therapeutic molecules [29].

To combine both advantages of liposome and polymeric micelles and achieve a biocompatible and biological stable materials, novel biomimetic block copolymers, cholesterol-end-capped poly(2-methacryloyloxyethyl phosphorylcholine) (CMPC), which have poly(MPC) as hydrophilic segment and cholesterol as hydrophobic segment were specially designed to be used as drug delivery system. Cholesterol (Chol) is one of the most common membrane sterols in animals that regulates membrane fluidity and plays an important role in self-association of molecules in biological systems [30,31]. It was found that Chol [32] had a strong tendency for self-association even if the contents of Chol in polymers were very low. Phosphorylcholine is an electrically neutral and zwitterionic head group. It represents the bulk of the phospholipid head groups present on the external surface of blood cells and is inert in coagulation assays. Biocompatibility of MPC based polymers had been well-documented in the literature [33–36].

We hypothesize here that polymers containing the Chol moiety and phospholipid moiety provide biomembrane mimicry and should be more compatible with the human body [37]. Furthermore, strong self-associative properties of Chol in the polymers ensure that drugs with a hydrophobic character can be easily incorporated into the core by a non-covalent bonding through hydrophobic interactions.

The current objective of this paper is to assess the possibility of construction of novel biomimetic micelles via self-assembly of novel biomimetic amphiphiles, CMPC. Fluorescence probe technique and transmission electron microscope (TEM) were used to characterize the micelles structure. Cell culture was used to probe into the cytotoxicity of this novel amphiphiles using commercial available amphiphiles, Cholesterol-end-capped PEO (CPEO), as controls. Anti-cancer drug adriamycin (ADR) was incorporated into the micelles by oil-in-water method. The release behavior of ADR from the nanosphere and the cytotoxicity of the drug-loaded micelles were examined. The novel biomimetic micelles with high biocompatibility may have great potential as polymer therapeutics for anti-cancer drug delivery.

2. Experimental section

2.1. Materials

Pyrene was purchased from Aldrich Co. and used as received. Cholesterol-end-capped PEO (CPEO20 and CPEO30) were kindly supplied by Nihon Emulsion Co., Ltd. Adriamycin (ADR) was kindly supplied by Zhejiang Hisun Pharmaceutical Co., Ltd. 2,2'-bipyridine (bpy) (AR, Hangzhou Chemical
Reagent Factory) and 2-bromoisobutyryl bromide (Aldrich, 98%) were used as received. The CuBr (AR, Shanghai No. 1 Chemical Reagent Factory) were purified by washing with glacial acetic acid, followed by absolute ethanol and ethyl ether, and then dried under vacuum. Cation exchange resin 732 was from Hangzhou Shuanglin Chemical Factory. Ethanol and 2-propanol were purchased from Huadong Medicine Co. Dichloromethane and triethylamine was refluxed with calcium hydride for 24 h before use. Other reagents were purified by conventional methods.

The novel biomimetic surfactant was prepared by Atom Transfer Radical Polymerization (ATRP) of MPC [38] as outlined in Scheme 1. The 10-Cholesteryloxydecanol (Chol) was converted into the ATRP macroinitiator (CholBr) through the reaction with 2-bromoisobutyryl bromide. This macroinitiator (CholBr) was then used to initiate the Atom Transfer Radical Polymerization of MPC and prepare the novel biomimetic surfactants (CMPC). The molecular weight of CMPC20, CMPC30 and CMPC50 were determined as 6350, 9460 and 15,360, respectively.

2.2. Preparation of micelles and drug-loaded nanoparticles

To a small glass vial, 10 mg of the CPEO or CMPC polymer were dissolved in 5 ml of water. This solution was stirred using a magnetic stir bar for 4 h and the micelle formatted directly in the solution.

Water-soluble ADR hydrochloride (1.0 mg) was solubilized in 2 ml of mixture of chloroform and triethylamine (2 ml of chloroform + 2.4 μl of triethylamine). The reaction between ADR hydrochloride and triethylamine resulted a water insoluble drug

Scheme 1. Reaction scheme for the synthesis of the block copolymers via Atom Transfer Radical Polymerization (ATRP) using macroinitiator route.
Adriamycin [39]. This solution was added dropwise to the aqueous micelle solution under vigorous stirring. The mixture was vigorously stirred at room temperature overnight open to atmosphere. Then micelle solution was then centrifuged at 12,000 rpm to eliminate unloaded adriamycin and aggregated particles. The drug loaded micellar solution was then used in the following experiments.

2.3. Fluorescence measurements

Pyrene was used as a hydrophobic fluorescent probe. Pyrene solution in acetone (4.8 × 10^{-4} M, 5 μl) was added to 4 ml of aqueous polymer solutions. The obtained samples containing pyrene (6 × 10^{-7} M) were kept for 24 h at 20 °C and sonicated for 5 min prior to measurement to allow complete evaporation of acetone. Fluorescence spectra were recorded on a spectrofluorometer (FP-770, Japan Spectroscopic) at room temperature. For fluorescence measurements, excitation spectra of pyrene were monitored at 394 nm. For determination of the critical micelle concentration (CMC) of the polymers, excitation spectra of pyrene were obtained at varying concentrations of the polymers, following the procedures reported by Wilhelm et al. [40].

2.4. Transmission electron microscope (TEM)

A drop of CPEO micelle solution containing 0.01% phosphotungstic acid was placed on a copper grid coated with carbon film, and dried at room temperature. The observation was carried out at 80 kV with JEM-1230 (Jeol, Japan).

2.5. Cell culture

MC3T3 osteoblast-like cells line derived from newborn mouse calvarium were chosen for cell culture studies. The MC3T3 cells were seeded into a 24-well plate at a density of 1.5 × 10^5 cells per well for 24 h after which the growth medium was removed and replaced with the medium containing nanoparticles. The medium used was α-minimum essential medium (α-MEM) containing 10% heat inactivated fetal calf serum (Sijiqin Biotech. Co., China), 80 U/ml penicillin and 100 U/ml streptomycin. The cells were incubated at 37 °C in a 5% CO₂ atmosphere.

2.6. Cell adhesion assay

The effect of nanoparticles on cell adhesion was determined with cell suspension incubated with or without nanoparticles. Cells were seeded with or without nanoparticles at a concentration of 0.2 mg/ml into a 96-well plate (in triplicate) at a cell density of 1.5 × 10^5 cells per well and incubated for 24 h at 37 °C in an atmosphere of 5% CO₂ in air (Forma Scientific Co.). Following incubation, the wells were washed twice with PBS to remove nonattached cells. The remaining cells were removed by digestion with 0.25% trypsin solution. The number of cells attached to the polymer surfaces was determined by hemocytometric counting. The mean value of five replicates was used as the final result for each sample. Cell attachment was expressed as a proportion of the number of cells attached to TCPS in the same culture media.

2.7. MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a simple non-radioactive colorimetric assay to measure cytotoxicity, proliferation or viability. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cells are able to convert this dye into a water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring [41]. Formazan crystals, then, can be dissolved in an organic solvent such as dimethylsulphoxide (DMSO). The amount of the Formazan in the solution could be quantified by measuring the absorbance of the solution at 550 nm and the resultant value is related to the number of living cells.

To determine cytotoxicity/viability, the MC3T3 cells were plated at a cell density of 1.5 × 10^5 cells per well in a 96-well plate and incubated at 37 °C in an atmosphere of 5% CO₂. After 72 h of culture, the medium in the well was replaced with the fresh medium containing micelles at the concentrations of 2 mg/ml. After 48 h, 200 l MTT solution (5 mg/ml in PBS) was added to each well, and incubated at 37 °C for 4 h for MTT formazan formation. After the medium and MTT were replaced by DMSO, the samples were incubated at 37 °C for additional 5 min to dissolve the MTT formazan. Then the plates were mildly shaken for 10 min to ensure the dissolution of formazan. The absorbency values were measured.
by using microplate reader (BIO-RAD, model 550) at wavelength 490 nm, blanked with DMSO solution. Five replicates were read for each sample, the mean value of the five was used as the final result. Cell activity was expressed as a proportion of the absorbency value of tissue culture polystyrenes (TCPS) in the same culture media.

2.8. Cell morphology observation

Fluorescein diacetate (FDA, Sigma) was dissolved in acetone to get 5-mg/ml stock solutions. The working solution was freshly prepared by adding 5.0 μl of FDA stock solution to 5.0 ml PBS.

To observe the cell morphology, the MC3T3 cells were plated at a cell density of 1.5 × 10^5 cells per well in a 96-well plate and incubated at 37 °C in an atmosphere of 5% CO₂. After 72 h of culture, the medium in the well was replaced with the fresh medium containing micelles at the concentrations of 2 mg/ml. After 4 h of incubation, 20 μl FDA working solution was added into each well and incubated for 5 min, the wells were then washed twice with PBS and confocal laser scanning microscopy (CLSM, BIO-RAD2000) examination carried out for cell morphology observation.

2.9. Determination of drug loading efficiency

The drug-loaded micelles were disrupted by the addition of ethanol and tetrahydrofuran (THF) (1:1, v/v), the amount of ADR entrapped was determined by measuring the UV absorbance at 319 nm. An ADR content entrapped into the cholesterol portion of micelles was calculated from the weight of the initial drug loaded micelle and the amount of drug incorporated from the following equation.

\[
\text{Drug loading efficiency (DLE) (\%) } = \frac{\text{amount of ADR in micelles}}{\text{amount of ADR - loaded micelles}} \times 100
\]

\[
= \frac{\text{ADR}}{\text{ADR} + \text{Polymer}} \times 100
\]

2.10. In vitro drug release studies

The in vitro drug release profiles of ADR from the CMPC micelles were determined as follows [42]. The appropriate amount of ADR loaded micelles were precisely weighted and suspended in 10 ml of a phosphate buffer solution (PBS, 0.1 M, pH 7.4). The micellar solution was introduced into a dialysis membrane bag and the bag was placed in 10 ml of phosphate buffer solution release media, and the media were stirred at 37 °C. At predetermined time intervals, 5 ml aliquots of the aqueous solution were withdrawn from the release media and another 5 ml fresh phosphate buffer solution was added into the release media. Then the concentration of ADR released was monitored using a UV spectrophotometer at 485 nm.

3. Result and discussion

Biomimetic surfactants on the base of cholesterol as hydrophobic segment and poly(MPC) as hydrophilic segment were used in the present study as drug delivery system. As a control, commercial available Cholesterol-end-capped PEO (CPEO) was chose to be used in cell culture to examine the cytocompatibility of these novel biomimetic amphiphiles. A summary of the various characteristics of the polymers is given in Table 1.

3.1. Fluorescence studies

The behavior of self-assembled micelle formation is investigated by fluorescence spectroscopy study. It was reported that the micelle formation of copolymers in water could be determined by fluorescence technique using pyrene as a hydrophobic probe and the critical micelle concentration (CMC) could be calculated from the fluorescence excitation spectra as pyr-

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mn</th>
<th>CMC (mg/mL)</th>
<th>Micelle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMPC20</td>
<td>6350</td>
<td>13.47 × 10⁻³</td>
<td>33.8 ± 3.0</td>
</tr>
<tr>
<td>CMPC30</td>
<td>9460</td>
<td>20.77 × 10⁻³</td>
<td>21.3 ± 3.5</td>
</tr>
<tr>
<td>CMPC50</td>
<td>15360</td>
<td>28.33 × 10⁻³</td>
<td>/</td>
</tr>
<tr>
<td>CPEO20</td>
<td>2300</td>
<td>3.24 × 10⁻³</td>
<td>33.3 ± 7.0</td>
</tr>
<tr>
<td>CPEO30</td>
<td>2800</td>
<td>3.64 × 10⁻³</td>
<td>26.4 ± 3.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated from the excitation spectra of pyrene as a function of polymer concentrations in water.

<sup>b</sup> Calculated from the TEM.

<sup>c</sup> Molecular weight from the ¹H-NMR results.

<sup>d</sup> Molecular weight from the feed ratio results.
ene partitions between aqueous and micellar environments [43].

Red shift of the excitation spectra of pyrene was observed with increasing concentration of CMPC with similar tendency of the reported copolymers [43]. The intensity ratio of $I_{339}/I_{334}$ versus log C of CMPC20 in the pyrene excitation spectra was plotted in Fig. 1. It is obvious that the curve was almost flat at the low concentration, and rapidly increased at the high concentration. This indicated the CMPC self-assembly to a micelle structure. The CMC was obtained from the intersection of two straight lines: the base line and the rapidly rising $I_{339}/I_{334}$ line. The estimated CMC values of CMPC20, CMPC30 and CMPC50 were $13.47 \times 10^{-3}$ mg/mL, $20.77 \times 10^{-3}$ mg/mL and $28.33 \times 10^{-3}$ mg/mL, respectively. The CMC values of the commercial amphiphile, CPEO20 and CPEO30, which were used as controls, were $3.24 \times 10^{-3}$ mg/mL and $3.64 \times 10^{-3}$ mg/mL, respectively.

It was not surprising to find that the CMC values of CMPC increased with the increase of the molecular weight of the CMPC. Since the hydrophobic part of each of the CMPC was the same with each other, when the molecular weight increased, the hydrophilic chain length increased. And the increase of the hydrophilic chain length depressed the aggregating tendency of the cholesterol segment. This brought the CMC to larger values. In any way, these CMC values were much smaller than those of low molecular weight surfactants and were comparable with those of other micelle-like polymer aggregates.

### 3.2. Microscopic characterization of the micelles

To further characterize the micelles and obtain direct visualization of size and morphology of the micelles (CMPC30), we investigated the micellar systems by transmission electron micrograph (TEM). Fig. 2 showed the result when the initial concentration of the CMPC30 micellar aqueous solution was 0.1 mg/mL. Obviously, the whole surface was uniformly covered by CMPC micelles. The shapes of the CMPC micelles were observed to be spherical shapes, and the diameters of these micelles ranged between 25 and 40 nm at a dehydrated state.

### 3.3. In vitro cytotoxicity measurements

Cell counts of the CPEO micelles and the CMPC micelles were measured with MC3T3 cells. Polymer concentration of the micelle solution was approximately 2 mg/mL. This concentration was above their CMC values (~0.01 mg/mL). Cell counts after 48 h of incubation with the micelles was shown in Fig. 3. This figure involved both the comparison of CPEO with...
CMPC at same concentration (2 mg/ml) and the comparison of CMPC with culture medium. The cell counts of CPEO20 and CPEO30 was less than 1000/ml, where the numbers of cells in the CMPC20 and CMPC30 samples were $1.257 \times 10^5$/ml and $1.292 \times 10^5$/ml. The cell counts showed no significant deference between CPEO20 and CPEO30 samples ($p > 0.05$) and between CPEO20 and CPEO30 ($p > 0.05$). On the other hand the cell counts of CMPC were significantly larger than those of the CPEO ($p < 0.05$). The numbers of cells in the CMPC samples were a little lower than that of the culture medium, but this difference was within standard deviation ($p > 0.05$).

Cytotoxicity of cells in CPEO micelles, CMPC micelles and culture medium after 48 h incubation time was determined using MTT method and the result is shown in Fig. 4. Both CPEO20 and CPEO30 exhibited considerable cytotoxicity ($p < 0.05$), while CMPC20 and CMPC30 revealed low cytotoxicity ($p > 0.05$). And the CMPC micelles showed approximate the same MTT values as that of free culture medium. These results confirmed that polymeric micelles of CMPC showed no cytotoxicity.

Fluorescence micrographs of the cells stained with FDA and corresponding phase contrast micrographs of MC3T3 cell line in pure culture media, CMPC20, CMPC30, CPEO20 and CPEO30 micelles containing medium at 4 h after seeding the cells were shown in Fig. 5. fluorescein diacetate (FDA) is a non-fluorescent fluorescein ester, which undergoes fluorochromacia in viable cells. This involved the penetration of FDA into the cell, its enzymatic hydrolysis that converted it to fluorescent fluorescein, and the accumulation of the latter in the cell [44]. In this paper, FDA were used to stain living cells while phase contrast micrographs were used to observe both the living and dead cells. The comparison between fluorescence micrographs of the cells stained with FDA and the corresponding phase contrast micrographs can provide valid index on the growing states of the cells. It can be seen from Fig. 5 that most of the MC3T3 cells were living and the cells grew very well in the pure culture media and in the media containing CMPC20 and CMPC30 micelles. This meant the cytotoxicity of the CMPC20 and CMPC30 micelles was very low and negligible. While in the case of the media that contained CPEO20 and CPEO30 micelles, cytotoxicity was obvious and almost no living cells could be found. It is clear that the biomimetic design of the molecular has a positive effect on the MC3T3 cells morphology.

It was not surprise to find that the CMPC showed low cytotoxicity if we considered the excellent biocompatibility of the zwitterionic phosphorylcholine group. It is believed that the biomimetic phosphorylcholine moiety has a strong hydrating property, also known as “free water”, which causes the hydrated phosphorylcholine group to act as a buffer to prevent biological molecules adsorption [45]. In this case, the ability of the biological molecules to interact with the phosphorylcholine group is limited, which results in a biocompatible material.

Low or no cytotoxicity of the blank polymeric micelles of CMPC demonstrated that these biomimet-
ic polymeric micelles could be used as healthy drug delivery system.

3.4. Determination of drug loading content

The drug loading efficiencies depending on the molecular weights of block polymer is shown in Table 2. The drug-loaded samples were prepared at fixed feed ratio of ADR to polymer and varied molecular weight of copolymer. As shown in Table 2, the drug loading efficiency did not change much with the molecular weights or hydrophilic chain lengths of block copolymers.

### Table 2

ADR\(^a\) loading contents of CMPC copolymer micelles

<table>
<thead>
<tr>
<th></th>
<th>CMPC20ADR</th>
<th>CMPC30ADR</th>
<th>CMPC50ADR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLE(^b)</td>
<td>3.6%</td>
<td>4.23%</td>
<td>4.03%</td>
</tr>
</tbody>
</table>

\(^a\) Adriamycin.

\(^b\) DLE means Drug Loading Efficiency (%).

3.5. Drug release behavior

The drug release behavior was demonstrated by plotting the relative release percentages of drug versus time in Fig. 6.

Since the initial burst effect was not observed in all examined samples, it confirmed that CMPC nanospheres could be prepared without any residual drug on their surfaces. While the free ADR exhibited rapid release behavior of above 80% within about 30 h, the ADR loaded into the inner core of nanospheres showed significant sustained release characteristics of less than 23% for 7 days.

As has been discussed above, the drug loading efficiency did not change much with the molecular weights or hydrophilic chain lengths of block copolymers.
polymers. We compared the release behavior of the CMPC30ADR, CMPC30ADR and CMPC50ADR sample to evaluate the effect of the molecular weight of the copolymer on the release behavior. It was clear that the rate of ADR released from the sample made of copolymer with a larger molecular weight was slower than that from the sample made of copolymer with a smaller molecular weight.

It is generally assumed that a drug is released by several mechanisms [46]: (a) Fickian diffusion through the polymer matrix, (b) diffusion through pores in the matrix, and (c) drug liberation by polymer erosion. ADR, owing to its moderate lipophilic character, is physically entrapped in hydrophobic core of micelles. And in our cases, degradation of the polymers was ignorable. Therefore, it is speculated that the drug release from nanoparticles is carried out mainly through Fickian diffusion.

Diffusion of a material in a polymer matrix is governed by the excluded volume and hydrodynamic interaction [42]. If we take into account that the hydrophobic parts of all the polymers were the same, we will come to the conclusion that the in vitro release behaviors of a lipophilic compound from these polymeric micelle systems are largely affected by its outer shell with hydrophilic properties. With the increase of the molecular weight of a copolymer, the hydrophilic segments in the polymer increased, resulting in the increase of the impedance of the diffusion of ADR through the polymer matrix. Consequently, in this micelle system with amphiphilic copolymer, the release rate of a drug is inversely proportional to the molecular weight of a block copolymer.

4. Conclusion

The CMPC copolymers which have poly(MPC) as hydrophilic segment and cholesterol as hydrophobic segment (CMPC) were successfully synthesized. The sizes of nanoparticles (micelles) prepared were within the range of 25–40 nm, and they were suitable for an injectable drug carrier. The nanoparticles prepared from the functionalized block copolymer had a very low CMC value, which suggested good stability of the nanoparticles. Results from cell culture declared that while CPEO showed obvious cytotoxicity, the biomimetic polymeric micelles CMPC demonstrated low or no cytotoxicity. The drug loading efficiency of nanoparticles did not change much with the hydrophilic chain length in the copolymers. While the free ADR exhibited rapid release behavior of above 80% within about 30 h, the ADR loaded into the inner core of nanospheres showed significant sustained release characteristics of less than 23% for 7 days. These experimental results suggested that the nanoparticles prepared from CMPC could be used as a good candidate for injectable drug delivery carrier.

Acknowledgment

This research was financially supported by National High Technology Research and Development Program of China (2001AA326030) and Natural Science Foundation of China (NSFC-20174035).

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