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Novel micelles from graft polyphosphazenes as potential anti-cancer drug delivery systems: Drug encapsulation and in vitro evaluation

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ABSTRACT

In this study, a new class of amphiphilic methoxy-poly(ethylene glycol) grafted polyphosphazene with glycine ethyl ester side groups (PPP-g-PEG/GlyEt) was synthesized and characterized. An anti-cancer agent doxorubicin (DOX) was encapsulated into polymeric micelles derived from those copolymers, which exhibited considerably strong impact on micelle morphology: turned the rod-like and spherical drug free micelles into spheres and vesicles respectively. The in vitro release behavior of those drug-loaded micelles exhibits a sustained release manner and is affected by drug content. Cytotoxicity assay against adriamycin-resistant human breast cancer MCF-7 cell line showed that drug-loaded micelles based on PPP-g-PEG/GlyEt micelles can effectively suppress cell proliferation and the cytotoxicity was both time and concentration related, an enhanced cytotoxicity was observed either with increasing drug concentration or with prolonged incubation time. Moreover, flow cytometry results revealed a particle size dependency in cellular uptake of drug-loaded micelles. These findings suggest that the present copolymers can encapsulate water insoluble anti-cancer agents and contribute to improve drug sensitivity of adriamycin-resistant cell line.

1. Introduction

Polymeric micelles, characterized as core-shell structured nano-size aggregations of amphiphilic polymer chains with hydrophobic inner core and hydrophilic corona, have aroused tremendous interest in pharmaceutical research. Generally, there are two main principles for polymeric micelles to conduct drug delivery. First the hydrophobic core of micelles can act as the reservoir of drugs and meanwhile the hydrophilic corona has the capacity to stabilize micelles. Secondly, polymeric micelles are small enough (<200 nm) to evade from bioclearance, therefore have long blood circulation time which helps drug-loaded micelles to reach the action site or conduct controlled drug release. Furthermore, compared with lipid-based vectors such as liposomes and lipid nanoparticles, synthetic polymeric micelles have brilliant advantages in molecule design. By controlling the composition and the length of polymer chains, a variety of sized and shaped micelles could be obtained, which develop towards advanced drug delivery systems. Currently, a majority of explorations in polymeric micelles for drug delivery focuses on cancer therapy. Based on the well-known enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986), drug-loaded micelles could be accumulated around tumors and perform high tumor inhabitant. For this aim polymeric micelles should be carefully designed to achieve the appropriate size, stability and drug loading efficiency under control (Nishiyama and Kataoka, 2006).

Polymer base is fundamental for the design of polymeric micellar drug delivery systems. Ideally polymers chosen for drug delivery should be biocompatible and can be easily tailored or functionalized. Polyphosphazene is a novel biocompatible and biodegradable inorganic polymer with two active chlorine side groups on each repeat unit. Compared with those popular biomaterials such as polycaprolactone (PCL) and poly(lactic acid) (PLA), the distinct advantage of polyphosphazene for drug delivery is the chlorine side groups on polyphosphazene can be readily substituted by other molecule/macromolecules, therefore, multi-functionalized polyphosphazenes with a variation of physical and chemical characteristics could be obtained (Singh et al., 2006). Pioneer researchers have fabricated several series of polyphosphazenes, and the biodegradation and biocompatibility of polyphosphazenes have been demonstrated (Andrianov et al., 2005). Other researchers further studied the biomedical application of polyphosphazenes such as nano-fibers (Nair et al., 2004) and hydrogels (Seong et al., 2006; Kang et al., 2006). Our group has been focusing on the micellization of amphiphilic polyphosphazenes and developed thermosensitive amphiphilic polyphosphazenes for local drug sustained release (Zhang et al., 2004, 2006). In present work, we aim to construct

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a micellar system based on amphiphilic polyphosphazenes for tumor targeting treatment. Herein the synthesis of a novel class of amphiphilic graft copolymer containing methoxy-poly(ethylene glycol) and glycine ethyl ester side groups (PPP-g-PEG/GlyEt) would be reported. The self-aggregation behavior of copolymers in water, drug encapsulation, in vitro release and cytotoxicity of anti-cancer agent-loaded micelles were investigated.

2. Materials and methods

2.1. Materials

Hexachlorocyclotriphosphazene (Acros Organics) was purified by sublimation at 80–90 °C. PEG (Fluka) was dried by azeotropic distillation with benzene before use. Aluminum chloride (99%) was purchased from Acros Organics and used without further purification. Pyrene (Acros Organics) was recrystallized twice from anhydrous ethanol before use. Doxorubicin (DOX) hydrochloride was kindly supplied by Juhua Group Pharmaceutical Factory (Zhejiang, China), and all other reagents were commercially available and used without further purification.

2.2. Methods

2.2.1. Synthesis and characterization of graft copolymer

Polyphosphazene backbones were synthesized by ring opening polymerization, then amino-terminal PEG_{2000} and glycine ethyl ester (GlyEt) were grafted onto backbones through nucleophilic substitution sequentially. The products were collected by precipitation in ether. Afterwards, crude copolymers were purified by dissolving precipitation twice and dialysis against pure water for 2 days. More synthesis details can be found in our previous reports (Zhang et al., 2004).

Composition of copolymers was confirmed by ^{1}H NMR spectra using an Avance DMX500 spectrometer using $CDCl_3$ as the solvent and trace tetramethylsilane (TMS) as an internal reference.

The critical micelle concentration (CMC) was determined by a fluorescence probe technique using pyrene as the fluorescent probe. The copolymer solutions with a varying range of concentration from 1.0×10^{-6} to 1.0 g/l were equilibrated with pyrene (3.0×10^{-7} M) for 10 h, then fluorescence measurement was carried out by a fluorescence spectrophotometer (FP 6000, Hitachi) at λ_{em} = 390 nm. Since bulk copolymers with high hydrophobic ratio are not readily dissolved in water, the dilute aqueous solutions of copolymers were firstly prepared by dialysis methodology. After lyophilized, the fluffy products were obtained which can be dissolved completely in water for CMC study.

2.2.2. Preparation methodology of DOX-loaded and unloaded micelles

Drug-loaded and blank micelles were prepared according to the same procedures. The copolymer was firstly dissolved in N,N-dimethylformamide (DMF) (for drug-loaded micelles, DOX was dissolved in DMF along with the copolymer in the presence of triethylamine (TEA)). Then the solution was allowed to stir for several hours achieving complete dissolution. Afterwards, a certain amount of pure water was added slowly (0.3 ml/min) under gently stirring, which induced micellization of amphiphilic copolymer. Finally, this micellar solution was dialyzed to remove DMF residue and a 0.45-μm microporous filter was used to eliminate any dust or unloaded DOX. The obtained micellar solution was lyophilized and properly stored before further characterization.

2.2.3. Determination of drug loading content in the micelles

The drug content encapsulated inside the micellar carrier was determined by the UV–vis spectrometry using the following standard method. Approximately 4 mg of freeze-dried micellar sample was dissolved in 10 ml of DMF, then the UV–vis absorption intensity at 483 nm was measured. The total amount of DOX-loaded was calculated from the established standard curve.

The drug content and the loading efficiency were calculated using the Eqs. (1) and (2) respectively:

$$\text{Drug loading content} = \frac{\text{mass of DOX encapsulated in micelles}}{\text{mass of DOX-loaded micelles}} \times 100\% \quad (1)$$

$$\text{Drug loading efficiency} = \frac{\text{mass of DOX encapsulated in micelles}}{\text{mass of DOX added}} \times 100\% \quad (2)$$

2.2.4. Dynamic light scattering (DLS)

The particle size and size distribution of various micelles were determined by DLS (90 Plus Particle Size Analyzer, Brookhaven Instruments Co.). The scattering angle was kept at 90° and the wavelength in vacuum was set as 658 nm during the whole experiment.

2.2.5. Transmission electron microscopy (TEM)

TEM images were recorded using a JEM 1230EX microscope operating at an acceleration voltage of 120 kV, and equipped with a Gatan CCD camera. To prepare the TEM samples, an aqueous solution of micelles was dropped onto a Formar-coated copper grid, excess micelle solution was gently removed using absorbent paper. The samples were allowed to dry under ambient conditions and conduct TEM observation without staining.

2.2.6. In vitro release profiles

Drug release experiments were conducted using the dialysis method. Typically, 1.0 ml of micellar solution in a concentration of 5 mg/ml was sealed in a dialysis bag (Spectrum MWCO 14000) and was immersed in 20 ml of phosphate buffer solution (PBS) at pH 7.4 and incubated at 37 °C. This outside buffer solution was periodically withdrawn and replaced by fresh PBS, and then the released drug content was determined by UV–vis spectrometry from the sampling PBS as described above.

2.2.7. Cell line and cytotoxicity evaluation

Adriamycin-resistant MCF-7 human breast cancer cell lines were seeded in a 96-well culture plate (5000 cells/well) and incubated for 24 h. After preincubation, the cell-culture medium was replaced with fresh medium. Free DOX, DOX-loaded and drug-free micelles at serial dilutions were added to the prepared cell plate respectively. At predetermined time, the media were discarded and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added. The plates were incubated at 37 °C for 4 h. Then the intracellular metabolized product MTT formazan was retrieved by addition of DMSO and incubation at room temperature for 10 min. The plates were read at 550 nm, and the cell viability was calculated. The DOX resistance of adriamycin-resistant MCF-7 cells was validated by cytotoxicity assay that its IC_{50} at 24 h is 16-fold higher compared to adriamycin-sensitive MCF-7 cell lines.

2.2.8. Flow cytometry

Fluorescence histograms were recorded with a BD FACSCalibur (Beckton Dickinson) flow cytometer and analyzed using Cell Quest software supplied by the manufacturer. Minimums of 10,000 events were analyzed to generate each histogram. Cells were washed and centrifuged before injected into the flow cytometer.

2.2.9. Fluorescence microscopy

Adriamycin-resistant MCF-7 cells were incubated for 3 h with the free DOX or DOX-loaded micelles at 37 °C; upon completion of

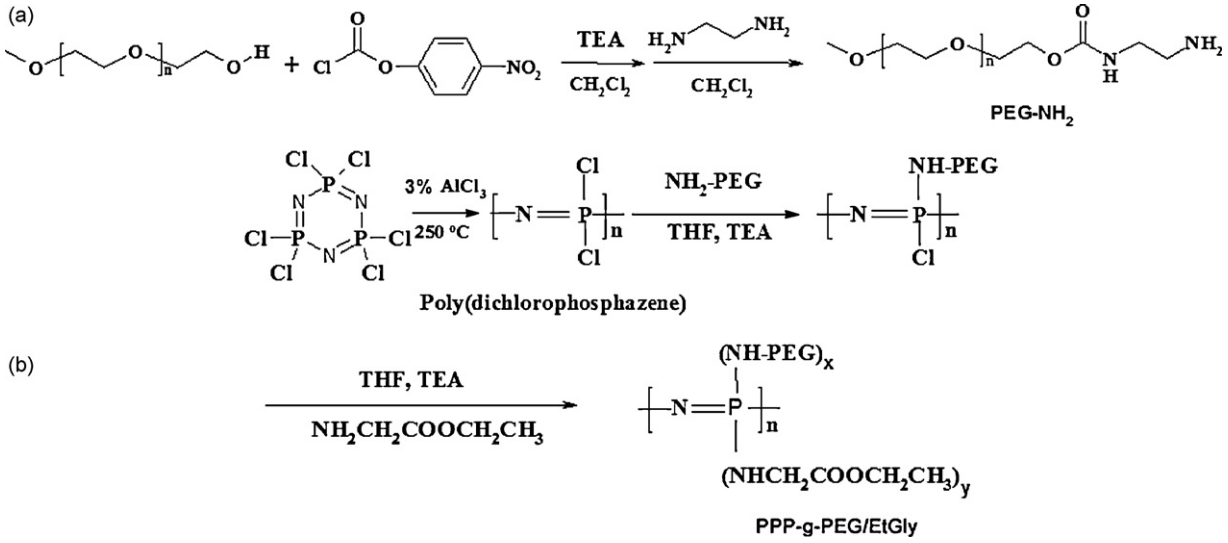

Fig. 1. Synthesis of PPP-g-PEG/GlyEt graft copolymers.

the incubation, cells were separated, washed and visualized with fluorescence microscope (OLYMPUS).

3. Results and discussion

3.1. Synthesis and characterization of graft copolymer

The synthesis of PPP-g-PEG/GlyEt graft copolymers involved the synthesis of a terminally functionalized PEG (Fig. 1a) and the final copolymers fabricated by substitute reaction between the terminal amino of PEG-NH_2/GlyEt and chlorine atoms as illustrated in Fig. 1b. Poly(dichlorophosphazene) dissolved in THF solutions were firstly reacted with PEG-NH_2, then excessive amount of GlyEt were added to make sure a complete substitution of chlorine atoms on the backbone. Three copolymers with different chemical compositions were prepared by changing the feeding ratio of PEG/GlyEt, which were abbreviated as ZP-1, ZP-2 and ZP-3 respectively. The unreacted GlyEt and PEG-NH_2 were eliminated by precipitation in diethyl ether and dialysis against pure water respectively.

Table 1
Molecular characteristics and blank micelles size of copolymers.

Polymer	Hydrophilic group ratio (w%)		Micelle size (nm)[a]	PDI
	Theoretical	Measured		
ZP-1	45	47	66	0.472
ZP-2	50	53	32	0.431
ZP-3	55	62	44	0.386

[a] Number average size.

The ^{1}H NMR spectra in Fig. 2 exhibit the signal of PEG and GlyEt, indicating the formation of PPP-g-PEG/GlyEt graft copolymer. The weight ratio of PEG fraction can be calculated from ^{1}H NMR data by comparing the peak intensities of the methylene protons of the ethylene oxide units of PEG at 3.6 ppm to the methylene protons of GlyEt at 1.1 ppm (Table 1). Increasing the feeding of PEG resulted in higher PEG content in the resultant polymer. Moreover, the measured hydrophilic group ratio was very close to the corresponding theoretical value, which illuminated the feasibility of this sequential substitute method for structure tailored graft polyphosphazenes.

The concentration dependence of the I_{338}/I_{333} ratios was illustrated in Fig. 3. The CMC value of ZP-1, ZP-2 and ZP-3 was calculated

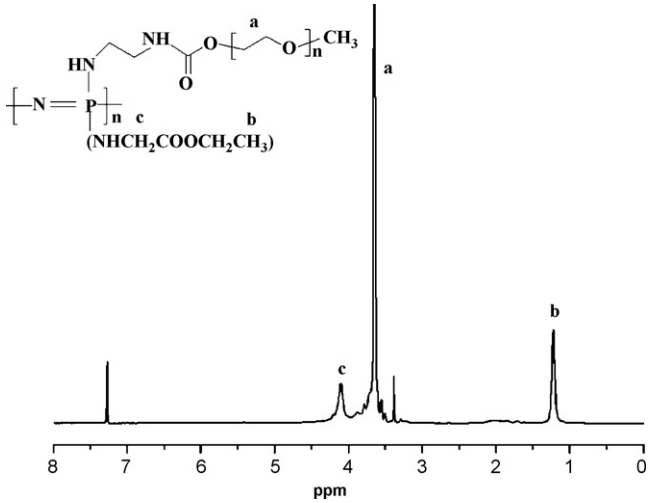

Fig. 2. ^{1}H NMR spectrum of the PPP-g-PEG/GlyEt graft copolymer in $CDCl_3$.

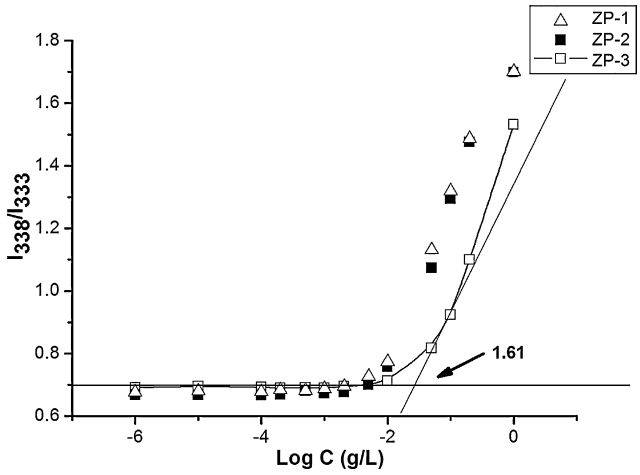

Fig. 3. CMC curves of three copolymers as determined from the excitation spectra.

Table 2
Characteristics of DOX-loaded micelles with different preparation conditions.

Polymer	Drug/polymer	DMF/water	Polymer concentration in DMF (mg/ml)	Size (nm)	PDI	Drug content (%)	Encapsulate efficiency (%)
ZP-1	3:10	1:1	10	277	0.032	16	70
ZP-2	3:10	1:1	10	273	0.070	17	74
ZP-3	3:10	1:1	10	228	0.010	19.5	85
ZP-3	2:10	1:1	10	279	0.083	12.3	74
ZP-3	1:10	1:1	10	217	0.155	6.3	70
ZP-3	2:10	1:1	3	147	0.146	8.4	50
ZP-3	2:10	1:3	10	164	0.138	9.6	58

as 0.0093, 0.012 and 0.024 g/l respectively, which agrees well with the copolymer compositions as the content of hydrophobic GlyEt groups in copolymers decreased as listed in Table 1.

3.2. Investigation of polymeric micelles

PEG grafted polyphosphazenes have been previously studied by Sohn group (Seong et al., 2006), however, the drug carriers from their polymers are in hydrogel or solid precipitate form. Here, polymeric micelles were produced through solvent dilution method. Typically, 30 mg of copolymer was dissolved in 3 ml of DMF, then 3 ml of deionized water was added at a rate of 0.3 ml/min under stirring. Dialysis was performed against deionized water for 10 h to remove DMF. For drug-loaded micelles, 9 mg DOX and 10 μl TEA were added together with copolymer and sonicated before water addition. The obtained DOX-loaded micelles were separated from insoluble-free DOX aggregates by filtration through 0.45 μm Waterman filter.

The size of micelles with various drug contents was measured by DLS and the results were listed in Tables 1 and 2. As shown in Table 1, the size of blank micelles increased with the increasing hydrophobic content. It is found that the drug incorporation induced significant size increase of DOX-loaded micelles compared to those of blank micelles, while no obvious size differences were found among DOX-loaded micelles with diverse drug content produced through same procedure (Table 2), which implies that drug content was not a dominant factor to influence micelle size. Table 2 also presents the relationship between particle size and preparation conditions, indicating that the initial polymer concentration in organic solvent and the amount of water added into organic solution before dialysis strongly influenced the particle size of drug-loaded micelles. The particle size would reduce by more than 40% either when the initial polymer concentration in DMF decreased from 10 to 3 mg/ml or with 3-fold water addition before dialysis.

Noteworthy, drug encapsulation remarkably reduced the particle size distribution index of polymeric micelles (Table 2), which favors the application of these micelles for drug delivery.

Polymeric micelles were visualized via TEM imaging. Dramatic morphology diversification appeared according to the weight ratio of hydrophilic PEG fraction. For the sample with 47% PEG fraction, rod-like micelles were observed (Fig. 4a), when the weight ratio of hydrophilic fraction increased to 53%, micelles with multiple morphology obtained, where the rods and spherical micelles are coexistent (Fig. 4b). In fact, DLS analysis of ZP-2 exhibits bimodal of size distribution (~23 nm for rods and ~164 nm for spheres), indicating that two different aggregations were anticipated. Due to smaller ones had higher weight ratio, the resultant number average particle size of ZP-2 was 32 nm (Table 1). When hydrophilic PEG weight ratio further increased to 62%, all micelles are spherical in the sample image (Fig. 4c).

Interestingly, the structure of DOX-loaded micelles is quite different from those blank micelles. Micelles of ZP-1 and ZP-2 metamorphosed into spheres (Fig. 4d and e), while the original spherical micelles from ZP-3 changed to vesicle like aggregates after drug encapsulation (Fig. 4f).

Theoretically, amphiphilic copolymers could assemble into rod-like micelles according to their physicochemical characteristics, there are several examples of rod-like micelles produced from amphiphilic copolymers (Elsabahy et al., 2007; Li et al., 2006), but none of them have observed the present rod to sphere transformation by introducing small molecules into micelles. In our previous work (Zhang et al., 2007), we reported a vesicle–sphere transformation using different polymer series with indomethacin (IND) as modal drug, where the hydro-bond interaction between IND and polymer was proposed inducing the vesicle formation. The similar phenomenon was also reported by Giacomelli et al. (2007). In present case, though no direct evidence which confirmed the suspected interaction forces between DOX and copolymer, it can be still concluded that drug encapsulation took the responsibility for those transfigurations. This assumption was supported by TEM analysis, which revealed a drug content dependency of micelle shape. As shown in Fig. 5, when the drug content of ZP-3 micelles is 6.3%, micelles still maintained their spherical shape (Fig. 5a), but as the drug content further increase to 12.3%, vesicles were observed (Fig. 5b).

3.3. In vitro release profile of DOX-loaded micelles

DOX release in vitro was studied in pH 7.4 or pH 5.5 PBS solutions at 37 °C. The freeze-dried micelles were dissolved in PBS (5 mg/ml), filtered through a 0.45-μm cellulose membrane and the DOX released was estimated by UV–vis spectrometry as described above. The results of release experiments are depicted in Fig. 6, a typical two-phase release profile was observed. Drug-loaded micelles underwent a fast initial release within first 8 h followed by a sustained drug release over next 5 days. The overall drug release rates of studied micelles at pH 7.4 were comparably slow, which implies those micelles have the potential for prolonged drug retention time in the blood circulation.

At pH 5.5, the release rates for DOX are much faster and a continuous release was observed after initial 10 h (Fig. 7). This is partly due to the promoted solubility of DOX at low pH, on the other hand, micellar dissociation also accelerated the release rate during the last few days, because the PPP-g-PEG/GlyEt graft copolymer have faster degradation rate in acidic media. This pH relative release is of particular interest in the context of tumor targeting. Based on the above results, it is anticipated that the majority of DOX will remain encapsulated within polymeric micelles in plasma after intravenous administration for a sufficiently long time favoring the accumulation of micelles in the vicinity of the tumor according to the EPR effect. Then, drug release will be accelerated by the relatively low local pH surrounding the tumor site or by the more acidic environment inside the endosome and lysosome of tumor cells after cellular uptake of micelles through endocytosis.

3.4. Cytotoxicity and cellular uptake studies

Adriamycin-resistant MCF-7 human breast cancer cells were exposed to copolymers over 24 h. No obvious suppression of cell viability was found within the measured concentration range

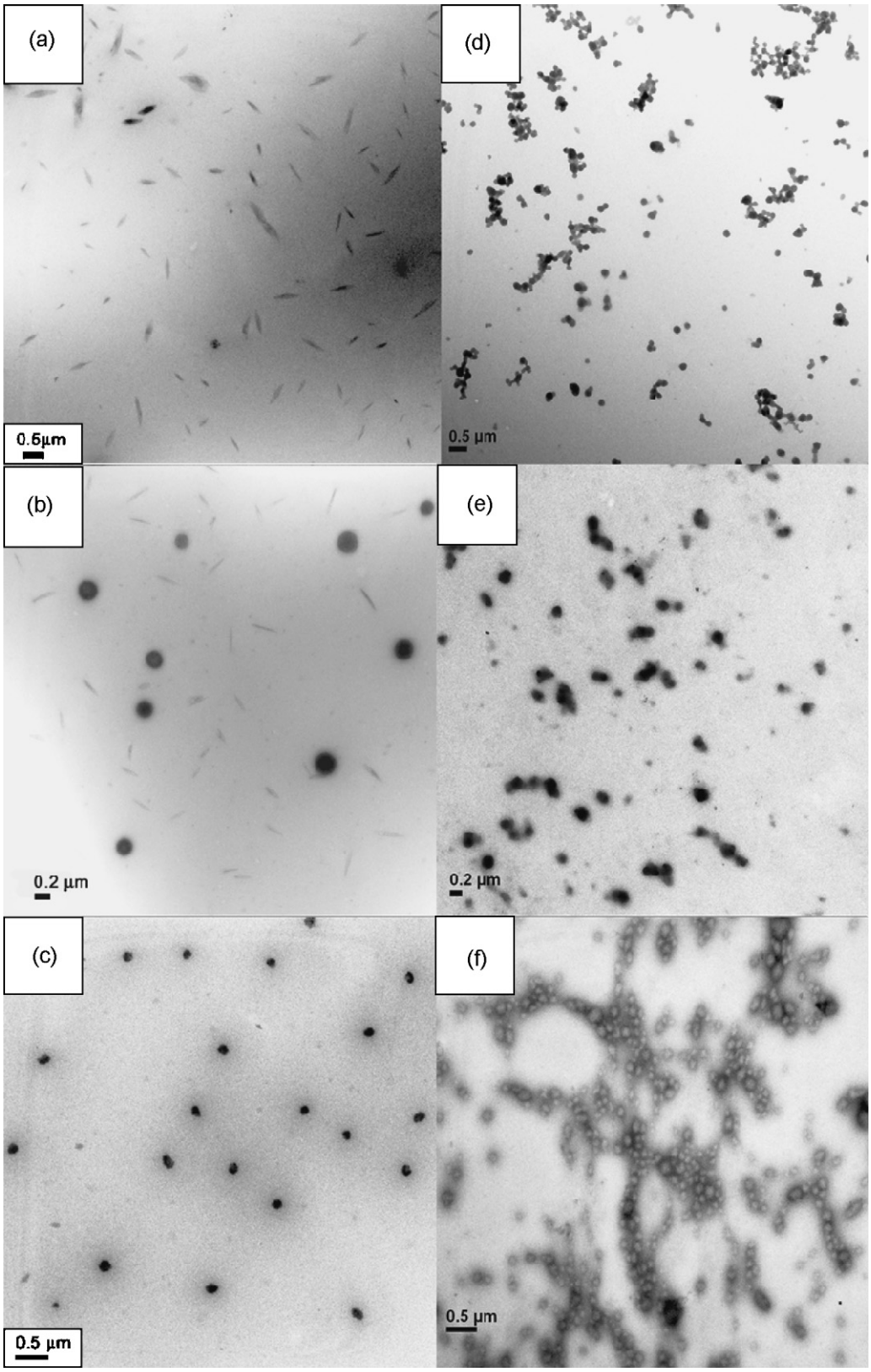

Fig. 4. TEM image of the blank micelles of ZP-1 (a), ZP-2 (b), ZP-3 (c) and of the DOX-loaded micelles of ZP-1 (d), ZP-2 (e), ZP-3 (f); samples were prepared in the same drug feed ratio (drug:polymer = 3:10).

(Fig. 8a) and the total cytotoxicity of PPP-g-PEG/GlyEt copolymers is as low as PEG-PCL copolymer (Cai et al., 2007) which is a popular polymeric material for drug delivery. This is not surprising since the polymer consisted of FDA approved PEO plus the degradable polyphosphazene and glycine ethyl ester. Therefore, this low toxicity of copolymer ensures that the cytotoxicity of drug-loaded micelles was induced by the DOX released from the micelles exclusively.

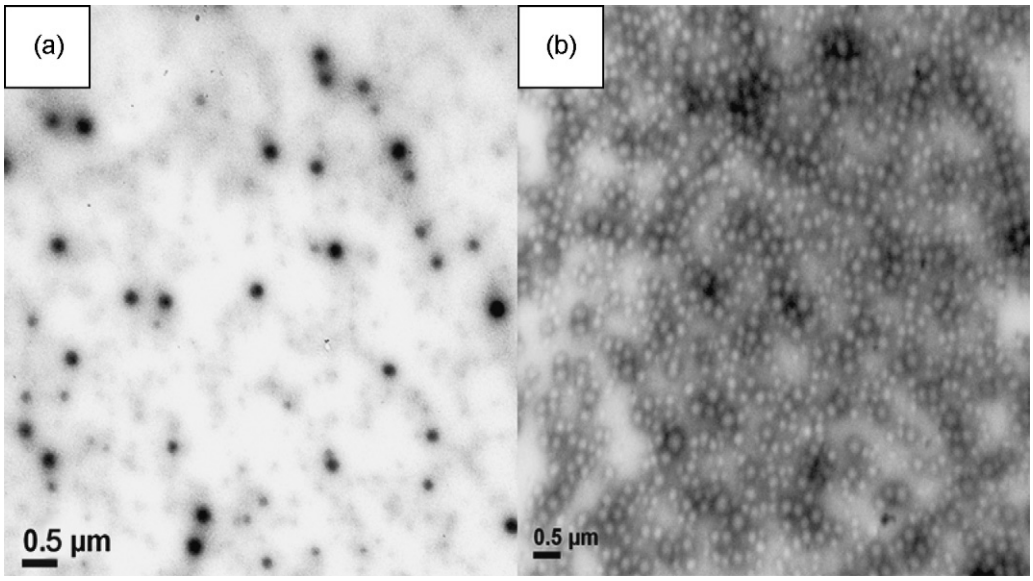

Fig. 5. TEM image of the DOX-loaded micelles of ZP-3 with drug feed ratio of 1:10 (a) and 2:10 (b) respectively.

Cell viability given in Fig. 8b revealed an effective suppression of cell proliferation by the treatment of DOX-loaded micelles. When the concentration of DOX encapsulated in micelles increased to 10 μg/ml, almost all the MCF-7 cell were killed after 72 h incubation. The cytotoxicity of ZP-1 micelles on MCF-7 cells is better than that of ZP-3 micelles and this is most probably attributed to the faster drug release rate of ZP-1 shown in Fig. 6. Both ZP-1 and ZP-3 micellar formulations showed the consistently improved cytotoxicity relative to delivery of free drug, the IC_{50}-cytotoxicity of DOX-loaded micelles are 3-fold more potent than free DOX (Fig. 8c). It was also observed that cytotoxicity of drug-loaded micelles is time related and the IC_{50} value at 72 h (Fig. 8d) was much lower than that of 24 h.

The uptake of DOX by adriamycin-resistant MCF-7 cells was monitored by flow cytometry. In these experiments, the cells were incubated with the solutions of drug-loaded micelles with equivalent DOX concentrations. The results shown in Fig. 9 indicated that the DOX were successfully transported into cells by being loaded in polymeric micelles. Much greater cellular uptake of DOX was observed in the samples incubated with micellar formulations than in that treated by free drug. Consistently, the direct observation of the cells by fluorescence microscopy revealed that the fluorescence intensity of micelle treated cells is an order of magnitude higher than cells treaded by free DOX (Fig. 10).

The above results may explain the mechanism of enhanced cytotoxicity of micelle formulations: first, a majority of the DOX-loaded micelles were transported into tumor cells by endocytosis since DOX release rate from micelles was relatively slow. Second, endocytosis of drug-loaded micelles is more efficient than the diffusion of drug molecules to go through cell membranes, which led to relatively higher intracellular DOX concentration in cells that treated by DOX-loaded micelles. Also the sustained drug release from micelles in cytoplasma was against the outside pumping action related to MDR effect and facilitated the retention of drug in the cell.

Noteworthy, flow cytometry analysis also reveal that the particle size of micelles has strong impact on the cell uptake efficiency, as shown in Fig. 9, adriamycin-resistant MCF-7 cells engulf more smaller micelles (147 nm) than bigger ones (279 nm), this result is consistent with other literatures concerning particle size and cellular uptake (Zauner et al., 2001; Hu et al., 2007), which implies that the smaller particle size of drug carrier not only favored the

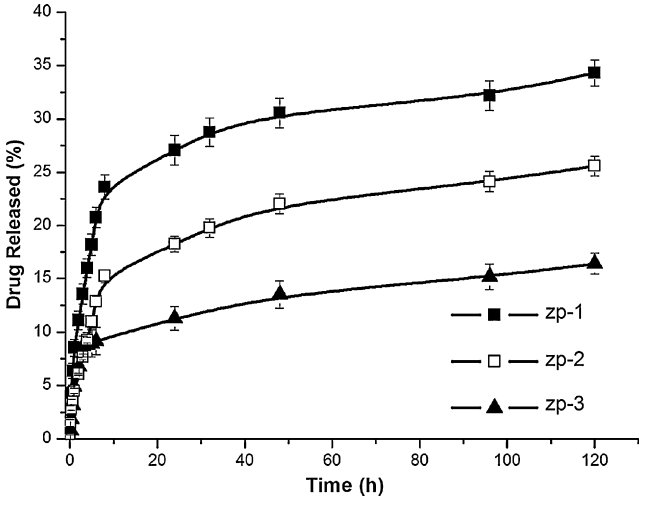

Fig. 6. Drug release profiles of different polymeric micelles in pH 7.4 PBS solutions at 37 °C, those micelles were prepared with same drug feed ratio (drug:polymer = 3:10) and the actual drug content were ZP-1 16%, ZP-2 17% and ZP-3 19.5%.

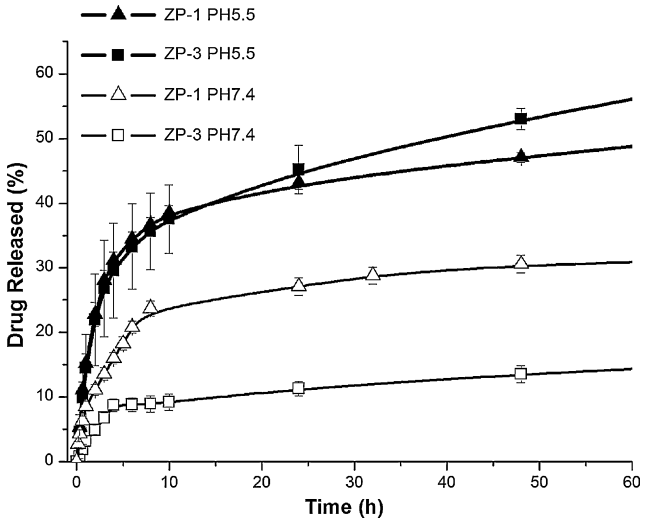

Fig. 7. In vitro DOX release profiles from ZP-1 and ZP-3 micelles with almost same drug content (~12%) in pH 7.4 (△, □) and pH 5.5 (▲, ■) PBS solutions, at 37 °C.

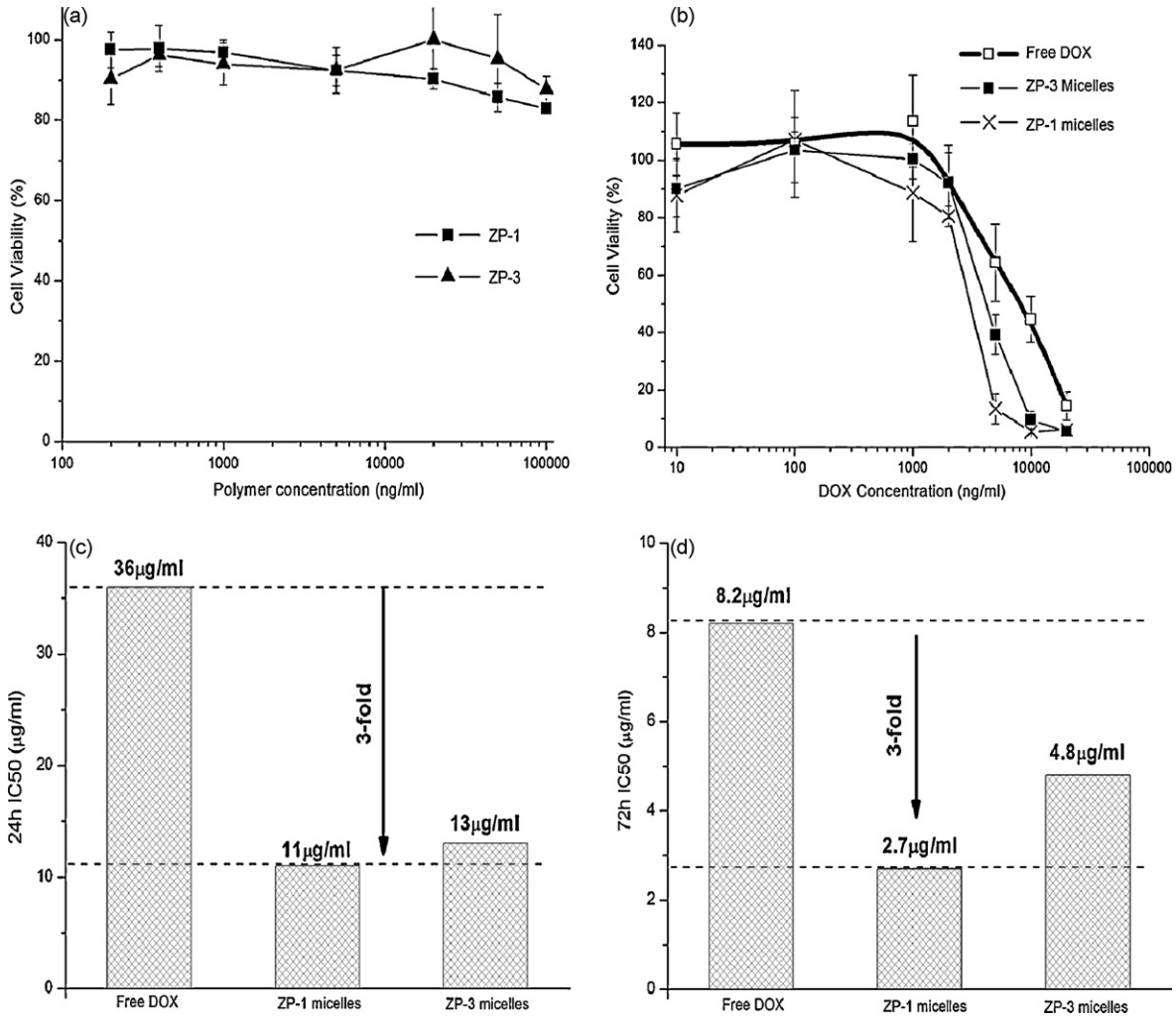

Fig. 8. Cytotoxicity study and IC_{50} evaluation of DOX-loaded micelles on adriamycin-resistant MCF-7 human breast cancer cells. (a) Cytotoxicity of copolymers only on MCF-7 cells; (b) 72 h cytotoxicity of different DOX formulations on MCF-7 cells; (c) comparison of 24 h IC_{50} of different DOX formulations; (d) comparison of 72 h IC_{50} of different DOX formulations.

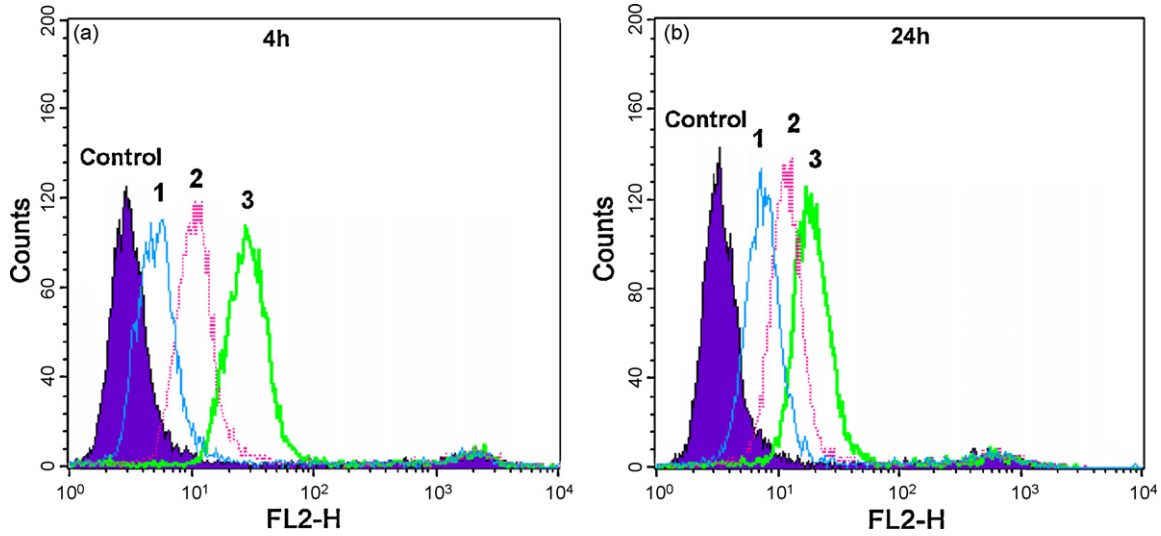

Fig. 9. Flow cytometry results of adriamycin-resistant MCF-7 human breast cancer cells treated by free DOX and drug-loaded ZP-3 micelles after 4 and 24 h incubation: (1) free DOX; (2) micelles with particle size of 279 nm and (3) micelle with particle size of 147 nm. (The preparation conditions of DOX-loaded micelles with different particle size were shown in Table 2.)

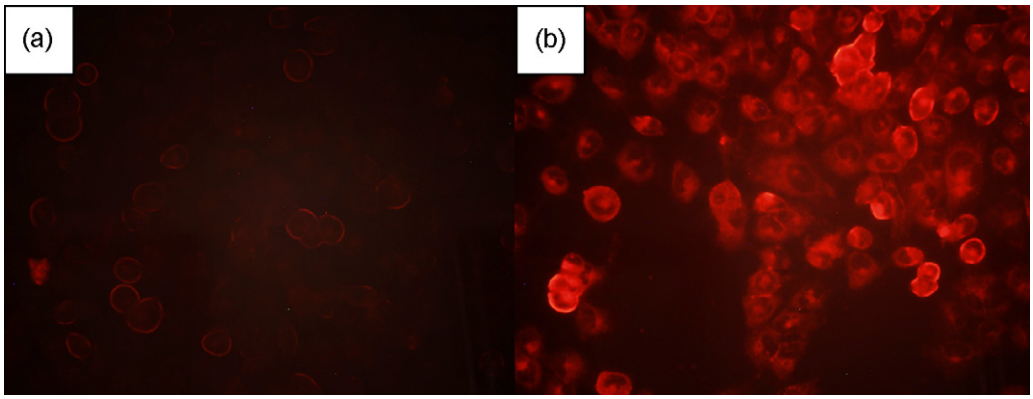

Fig. 10. Fluorescence micrographs of adriamycin-resistant MCF-7 human breast cancer cells incubated for 4 h incubation with free DOX (a) and with DOX-loaded ZP-3 micelles (b) at equivalent DOX concentration.

accumulation of carriers to tumors but also enhanced the cellular internalization efficiency.

4. Conclusion

Amphiphilic PPP-g-PEG/GlyEt copolymers can self-assembled into spherical and rod-like micelles in water with diameters ranging from 50 to 130 nm according to the hydrophilic ratio of different copolymers. These micelles can encapsulate DOX with high drug content (>10%) and high drug encapsulation efficiency (>70%). The shape of drug-loaded micelles was strongly influenced by drug content. The original rod-like micelles of copolymers with lower hydrophilic ratios converted into spherical nanoparticles after drug encapsulation, while the spherical micelles of copolymer with higher hydrophilic ratio changed into vesicles when the drug content is high enough (>12%). Drug-loaded micelles were quite stable and can be freeze-dried for long-term storage. The pH-related drug release behavior can ensure drug retention inside the micelle during blood circulation, and sustained release in cells for therapy. Further studies on in vitro cytotoxicity of drug-loaded micelles upon the adriamycin-resistant MCF-7 cells exhibit enhanced cell growth inhibition compared to free DOX. From the results of flow cytometry assay, we can also conclude that micelles with smaller particle size will be more efficient to transport drug into tumor cells. Accordingly, this new series of polyphosphazene seem to be promising for anti-cancer drug delivery. In vivo pharmacokinetics and pharmacodynamics of these drug vehicles is still under going.

Acknowledgement

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