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Biodegradable vesicular nanocarriers based on poly(ε-caprolactone)-*block*-poly(ethyl ethylene phosphate) for drug delivery

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ABSTRACT

Biodegradable polymer vesicle for drug delivery is reported. Poly(ε-caprolactone)-*block*-poly(ethyl ethylene phosphate) with well-defined structure (PCL_{150}-*b*-$PEEP_{30}$) has been prepared by ring-opening polymerization. It forms vesicles in aqueous solution using the thin-film hydration method and further exclusion of the as-formed vesicles results in vesicles at nano-size, demonstrated by confocal laser scanning microscope (CLSM) and transmission electron microscopy observations. Doxorubicin (DOX) has been loaded into the vesicles with a loading content of 4.38% using an acid gradient method. The release of DOX from the vesicles is accelerated in the presence of an enzyme phosphodiesterase I that is known to catalyze the degradation of polyphosphoester, achieving 83.8% release of total loaded DOX in 140 h. The DOX-loaded vesicles can be successfully internalized by A549 cells, and it results in enhanced inhibition to A549 cell proliferation, likely owning to the sustained intracellular release of DOX as observed by CLSM. With these properties, the vesicles based on the block copolymer of PCL and PEEP are attractive as drug carriers for pharmaceutical application.

1. Introduction

Polymer vesicles based on block copolymers with a hydrophobic wall and a hydrophilic corona have attracted considerable attention due to the enhanced toughness and reduced permeability compared with the lipid vesicles [1–3]. Various polymer-based vesicular systems have been reported [4–9], and they have shown potential in a variety of applications such as artificial organelle, artificial cell, cellular hemoglobin-based oxygen carriers, DNA vectors, nanocontainers or nanoreactors [6,10–14]. Like many other nanocarriers which are potential for drug delivery [15–19], polymer vesicles are also promising for drug delivery by encapsulation of hydrophilic guest drug molecules in the aqueous lumen of the vesicles and hydrophobic drugs in the core of the vesicles simultaneously. For example, Discher's group has reported biocompatible and biodegradable vesicles based on block copolymer of poly(ethylene oxide) (PEG) and poly(lactic acid) for simultaneous delivery of paclitaxel and doxorubicin [20]. In addition, attention on polymer vesicles for drug delivery has also been paid on those assembled from amphiphilic block polypeptides other than block copolymers of polyesters and poly(ethylene oxide) [21,22]. Drug release profile from polymer vesicles can be adjusted or controlled by various environmental factors, including oxidation [7,10,23], pH alternation [22,24–26], temperature change [21,27,28].

Polyphosphoesters (PPE) is a series of polymers with phosphoester linkages in the backbone. The biocompatibility, biodegradability and structural flexibility of PPE render them potential for drug delivery [29–31]. With convenient methods based on ring-opening polymerization of cyclic phosphoester monomers [32,33], we have previously synthesized structurally and compositionally defined block copolymers of polyphosphoester with PEG or aliphatic polyesters [32,34,35], and have also observed that triblock copolymers of poly(ethyl ethylene phosphate) and poly(ε-caprolactone) (PCL) or polylactide are amphiphilic, which are able to self-assemble into micellar nanoparticles with hydrophilic shell of poly(ethyl ethylene phosphate) (PEEP) in aqueous solution [29,35,36]. We have also demonstrated that with surface conjugation of galactosamine, micellar nanoparticles from diblock copolymer of PCL and PEEP (PCL_{67}-*b*-$PEEP_{36}$) can be used as drug carriers for targeted drug delivery to HepG2 cells [29].

The *in vitro* degradation studies reveal that PPE or its copolymers are biodegradable under enzymatic catalysis of phosphodiesterase I, which is present in human cancer cells [37]. Such an enzymatic catalyzed degradation may be useful for the design of

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vesicular system for controlled drug release, particularly for intracellular drug delivery to cancer cells. In this study, we have synthesized diblock copolymer of PCL and PEEP with well-defined structure (PCL_{150}-*b*-$PEEP_{30}$). It forms vesicles in aqueous solution by a thin-film hydration method and further exclusion of the as-formed vesicles results in vesicular nanocarriers. Doxorubicin has been loaded into the nanocarriers, and the drug release with response to enzyme phosphodiesterase I has been studied. The internalization of DOX-loaded vesicular nanocarriers by A549 cells and the inhibition to A549 cell proliferation have also been investigated *in vitro*.

2. Experimental section

2.1. Materials

ε-Caprolactone (CL) (Acros Organics, 99%) was dried over calcium hydride for 48 h at room temperature, followed by distillation under reduced pressure just before use. 2-Ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP) was synthesized according to the literature [38]. Aluminum isopropoxide ($Al(O^iPr)_3$), mainly composed of trimer (A_3), was obtained according to the literature [39]. Stannous octoate ($Sn(Oct)_2$, Sinopharm Chemical Reagent Co., Ltd., China) was purified according to a method described in literature [40]. Triethylamine was refluxed with phthalic anhydride, then with potassium hydroxide, and distilled. Toluene was refluxed over sodium. Tetrahydrofuran (THF) was dried over potassium-sodium and distilled just before use. Milli-Q water (Millipore Milli-Q synthesis, 18.2 MΩ) was prepared using a Milli-Q Synthesis System (Millipore, Bedford, MA, USA). The anticancer drug doxorubicin hydrochloride (DOX) is a product of Zhejiang Hisun Pharmaceutical Co, Ltd. Phosphodiesterase I from Crotalus atrox and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. PKH26 were obtained from Sigma Chemical Co. All other solvents and reagents were used as received.

2.2. Synthesis of poly(ε-caprolactone) (PCL_{150}-OH)

To ε-caprolactone (4.60 g, 40 mmol, 1 mol L^{-1} in toluene) in a fresh flamed and nitrogen purged round-bottom flask was added designed amount of A_3 of $Al(O^iPr)_3$ (188 μmol in 85 μL of THF) in a glove box (H_2O and O_2 contents <0.1 ppm). The solution was stirred at 25 °C for 1 h. Then the solution was deactivated with 20-fold excess acetic acid and precipitated into excess diethyl ether. The precipitate was dried under vacuum to a constant weight at room temperature to obtain the product. The yield was approximately 70%. The polymerization degree of the PCL macroinitiator was 150, which was calculated based on the integration ratio of the triplet resonance at 4.03 ppm (2H) and the singlet resonance at 3.66 ppm (2H) from its ^1H NMR. The molecular weight distribution of PCL_{150}-OH was 1.16 which was determined by GPC measurement as described below.

2.3. Synthesis of poly(ε-caprolactone)-block-poly(ethyl ethylene phosphate) (PCL-b-PEEP)

Block copolymers were obtained by ring-opening polymerization of EEP using PCL_{150}-OH as the initiator and $Sn(Oct)_2$ as the catalyst. As an example, $Sn(Oct)_2$ (0.030 g, 0.073 mmol) was added to a solution of EEP (0.90 g, 5.9 mmol) and PCL_{150}-OH (2.50 g, 0.146 mmol) in THF at 30 °C. After 3 h reaction, the mixture was concentrated and the residue was precipitated in diethyl ether/methanol (10/1, v/v) twice. The obtained block copolymer was dried under vacuum to a constant weight at room temperature with a yield of 75%.

2.4. Characterization of polymers

Bruker AV300 NMR spectrometer was used for ^1H, ^{13}C NMR spectra measurements. Deuterated chloroform ($CDCl_3$) containing 0.03 v/v% tetramethylsilane (TMS) was used as the solvent. Gel permeation chromatography (GPC) system was composed of a Waters 1515 pump and a Waters 2414 refractive index detector equipped with Waters Styragel® High Resolution columns (1 × HR4, 1 × HR2 and 1 × HR1, effective molecular weight range 5000–500,000, 500–20,000, 100–5000, respectively). Chloroform was used as mobile phase at the flow rate of 1 mL min^{-1} at 40 °C. Molecular weights and molecular weight distributions were analyzed using Waters Breeze software. Monodispersed polystyrene standards were used to generate the calibration curve. Samples (4 mg mL^{-1}) were diluted with 1 mL of chloroform and analyzed.

2.5. Preparation of PCL_{150}-b-$PEEP_{30}$ vesicles by thin-film hydration

PCL_{150}-*b*-$PEEP_{30}$ (5.0 mg) was dissolved in 500 μL of THF in a round glass flask. Evaporation of the solvent under rotation yielded thin film on the side of flask. The residual THF was removed from the film under vacuum overnight at room temperature. The polymer thin film was then hydrated in 10 mL of either Milli-Q water (Millipore Milli-Q system) or phosphate buffered saline (PBS, pH 7.4, 0.01 M), or citric acid buffer (pH 4.0, 0.1 M) with simultaneous sonication for at least 30 min, and then heated at 65 °C for at least 12 h.

2.6. Characterization of vesicles

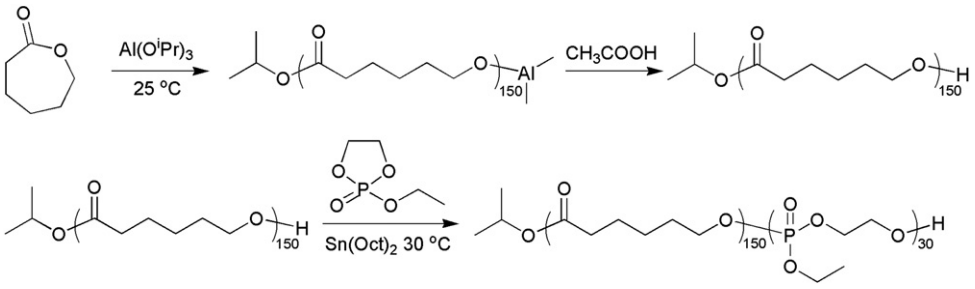
Optical microscopy observation was performed under Nikon TE2000-U inversed fluorescence microscope. Confocal laser scanning microscopy was performed with a Zeiss LSM510 confocal laser scanning microscope imaging system (Germany) with an upright confocal microscope. The data was analyzed by LSM 5 Image Examiner software.

Transmission electron microscopy (TEM) was performed on a JEOL-2010 transmission electron microscope with an accelerating voltage of 200 kV. The samples were prepared by pipetting a drop of vesicles in aqueous solution (0.25 mg mL^{-1}) onto a 230 mesh copper grids coated with carbon and allowing drying in air before measurements. The vesicles were extruded through 100 nm polycarbonate membranes (Avestin Inc., Ottawa, Canada) in an extruder system (LiposoFast Basic; Avestin Inc.) before measurements.

The size and size distribution of the vesicles were measured by dynamic light scattering (DLS) carried out on a Malvern Zetasizer Nano ZS90 with a He–Ne laser (633 nm) and 90° collecting optics. Sample was prepared at a concentration of 0.25 mg mL^{-1}. Measurements were carried out at 25 °C, and data was analyzed by Malvern Dispersion Technology Software 4.20.

2.7. Loading and release of doxorubicin

Doxorubicin in hydrochloride salt form (DOX) was loaded into vesicles by a pH-gradient method. The as-formed vesicles of PCL_{150}-*b*-$PEEP_{30}$ in citrate buffer (pH 4.0) via membrane hydration were extruded through 100 nm polycarbonate membrane (Avestin Inc., Ottawa, Canada) using the LiposoFast Basic extruder (Avestin Inc.) at 65 °C. The pH gradient is created across the vesicle membrane upon dialysis of the excluded vesicles in phosphate buffered saline (PBS, pH 7.4, 0.01 M) at 4 °C. After changing the dialysis solutions



Scheme 1. Synthesis procedure of diblock copolymer PCL_{150}-*b*-$PEEP_{30}$.

every 2 h for 4 times, the extruded vesicles were incubated with DOX in PBS at a weight ratio of 10:1 (polymer to drug) for 12 h at 65 °C. The unencapsulated DOX was removed by dialysis (MWCO, 100 KDa) at 4 °C in PBS. In order to calculate the total DOX encapsulated in the vesicles, HPLC grade acetonitrile (3 mL) was added to 1 mL of the dialyzed vesicle solution to disrupt the DOX-loaded vesicles completely. The concentration of DOX encapsulated in the vesicles was then measured by high performance liquid chromatography (HPLC) as described below.

Release of DOX was performed in duplicate. The DOX-loaded vesicles (0.5 mL) and phosphodiesterase I (0.25 mg mL^{-1} in 0.5 mL of PBS, 5 Unit L^{-1}) were mixed. The mixture was supplemented with $MgCl_2$ at 5 mM, and then enclosed in a dialysis bag (MWCO, 14 KDa). It was further incubated with 12 mL of PBS with 5 mM $MgCl_2$ at 37 °C under orbital shaking. The DOX-loaded vesicles without phosphodiesterase I was examined simultaneously as a control. At predetermined time intervals, all external solution outside the dialysis tubing was collected and equal volume of fresh medium was added. DOX concentration in the release medium was determined by HPLC measurements as described below.

2.8. Determination of DOX concentration

HPLC analyses were performed using a Waters HPLC system consisting of Waters 1525 binary pump, Waters 2475 fluorescence detector, 1500 column heater and a Symmetry C18 column. HPLC grade acetonitrile/water (50/50, v/v) with pH 2.7 adjusted by $HClO_4$ was used as the mobile phase at 30 °C with a flow rate of 1.0 mL min^{-1}. Fluorescence detector was set at 460 nm for excitation and 570 nm for emission and linked to Breeze software for data analysis. Linear calibration curves for concentrations in the range of 0.098–100 μg mL^{-1} were constructed using the peak areas by linear regression analysis.

The drug loading content (DLC) and efficiency (DLE) were calculated by the following equations:

$$\mathrm{DLC\%} = \frac{\text{amount of DOX in vesicles}}{\text{amount of DOX} - \text{loaded vesicles}} \times 100\%$$

$$\mathrm{DLE\%} = \frac{\text{amount of DOX in vesicles}}{\text{total amount of DOX for drug loading}} \times 100\%$$

2.9. Cell internalization studies

A549 cells (5×10^4 cells per well) were seeded on coverslips in a 24-well tissue culture plate. After 24 h culture, DOX-loaded vesicles or DOX in free form dissolved in complete RPMI 1640 culture medium with equal amount of DOX (10 μg mL^{-1}) were added to distinct wells and the cells were incubated at 37 °C. After washing with PBS, cells were stained with Hoechst 33342 at

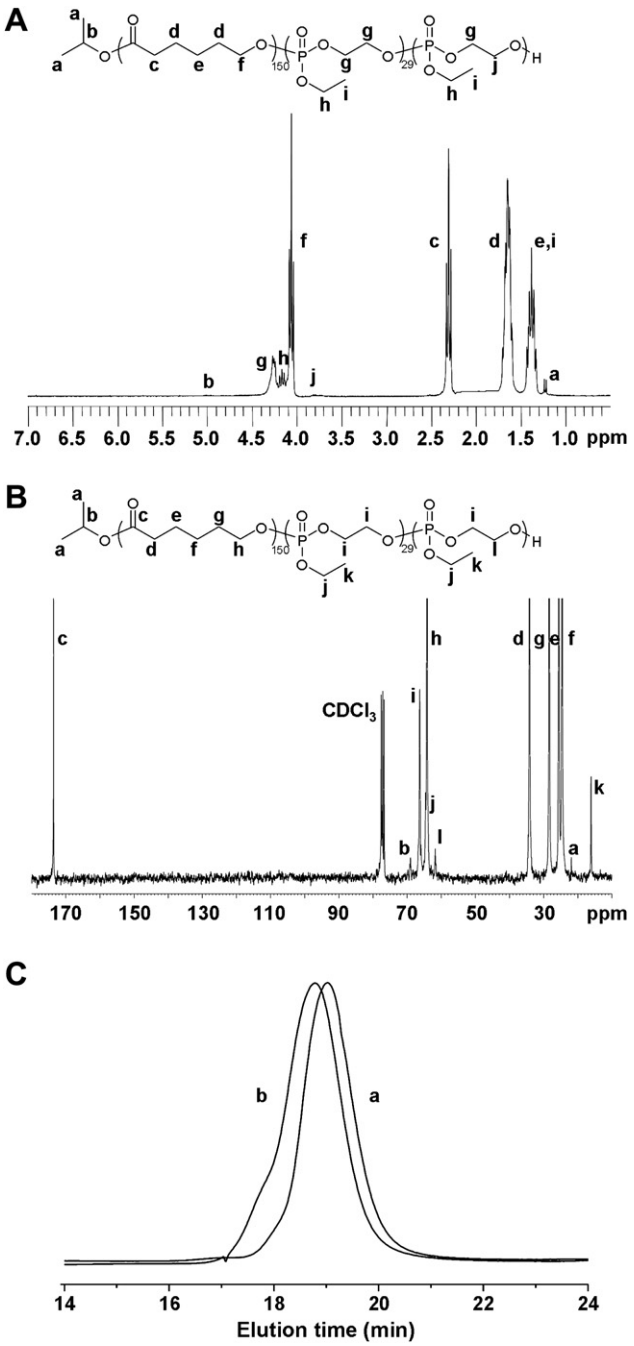


Fig. 1. ^1H NMR (A), ^{13}C NMR (B) spectra of PCL_{150}-*b*-$PEEP_{30}$ in $CDCl_3$, and GPC chromatograms of PCL_{150}-OH (a) and PCL_{150}-*b*-$PEEP_{30}$ (b) (C).

1×10^{-2} mM for 15 min, then the cells were fixed with 4% formaldehyde and the slides were mounted and observed with a Zeiss LSM510 Laser Confocal Scanning Microscopy imaging system.

2.10. Cell proliferation assay

The relative cytotoxicity of the vesicles was assessed with a methyl tetrazolium (MTT) viability assay against A549 cells. DOX in free form was used as a control. The cells were seeded in 96-well plate at 10,000 cells per well in 100 μL of RPMI 1640 culture medium containing 10% fetal bovine serum, and incubated at 37 °C in 5% CO_2 humidified atmosphere for 24 h. The medium was then replaced with 100 μL of DOX or DOX-loaded vesicles in complete culture medium at different DOX concentrations from 0.8×10^{-3} to 4 μg mL^{-1}. The cells were further incubated for 72 h, then 25 μL of MTT stock solution (5 mg mL^{-1} in PBS) was added to each well to achieve a final concentration of 1 mg mL^{-1}, with the exception of the wells as blank, to which 25 μL of PBS was added. After incubation for another 2 h, 100 μL of extraction buffer (20% SDS in 50% DMF, pH 4.7, prepared at 37 °C) was added to the wells and incubated overnight at 37 °C. The absorbance was measured at 570 nm using a Bio-Rad 680 microplate reader. The cell viability was normalized to that of A549 cells cultured in the complete culture medium.

3. Results and discussion

The block copolymer PCL_{150}-*b*-$PEEP_{30}$ was synthesized according to the procedure we reported through ring-opening polymerization of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP) using alcohol and $Sn(Oct)_2$ co-initiation system [33]. As illustrated in Scheme 1, hydroxyl end-capped PCL with 150 repeated units (PCL_{150}-OH) was firstly synthesized by ring-opening polymerization of ε-caprolactone (CL) under the initiation of aluminum isopropoxide ($Al(O^iPr)_3$), which was followed by deactivation by acetic acid [41]. EEP was then polymerized in the presence of PCL_{150}-OH and $Sn(Oct)_2$. The diblock copolymer was isolated by concentrating the polymerization mixture and precipitating it in diethyl ether/methanol (10/1, v/v). GPC analyses shown in Fig. 1 revealed that the molecular weight distribution of block copolymer is narrow (PDI = 1.26, with M_n of 31800) though a small high molecular weight shoulder was observed, due to the side reaction of polyphosphoester as earlier described [33]. According to its ^1H NMR spectrum (Fig. 1), the degree of polymerization (DP) of EEP was calculated to be 30 based on the integration ratio of resonances at 4.10–4.30 ppm and at 2.30 ppm. The molecular weight calculated based on ^1H NMR is 21670, which is lower than that measured by GPC analysis using polystyrenes as the standards, but is in agreement with the phenomenon observed by our earlier studies [32].

To demonstrate that PCL_{150}-*b*-$PEEP_{30}$ forms vesicular structure in aqueous solution, the block copolymer was dissolved in THF. Evaporation of the solvent yielded in thin film of PCL_{150}-*b*-$PEEP_{30}$. After removing the residual THF under vacuum overnight at room temperature, the thin film was hydrated in either Milli-Q water or

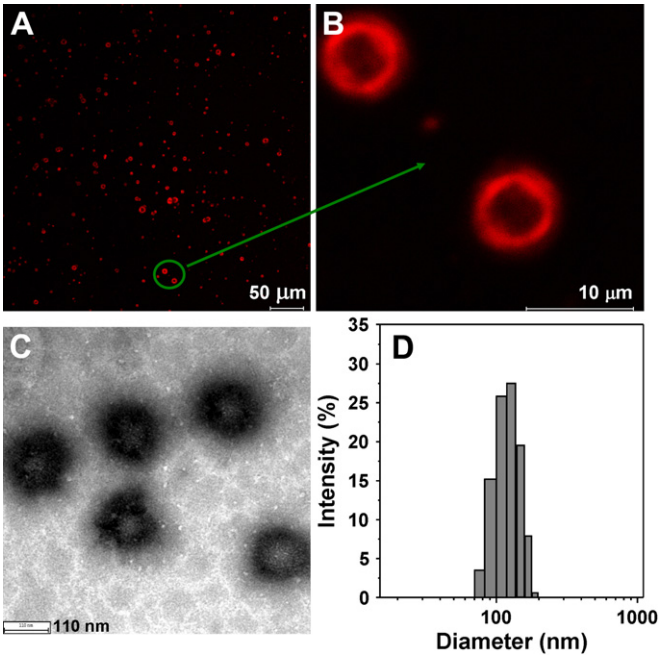


Fig. 2. CLSM image of the as-formed micron-sized vesicles of PCL_{150}-*b*-$PEEP_{30}$ stained with PKH26 in Milli-Q water (A) and its magnified image (B); Transmission electron microscope image of PCL_{150}-*b*-$PEEP_{30}$ vesicles that was excluded through a 100 nm polycarbonate membrane (C) and size distribution of excluded vesicles of PCL_{150}-*b*-$PEEP_{30}$ measured by dynamic light scattering (D).

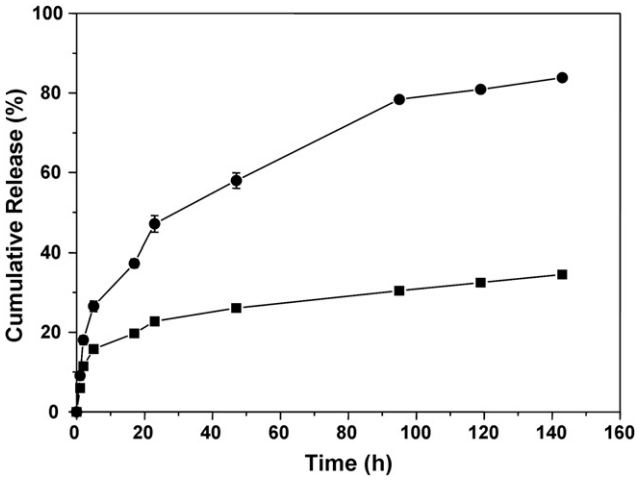


Fig. 3. Cumulative release of DOX from PCL_{150}-*b*-$PEEP_{30}$ vesicles without (■) or with (●) phosphodiesterase I in phosphate buffered saline (pH 7.4, 0.01 M, with 5 mM Mg^{2+}) at 37 °C.

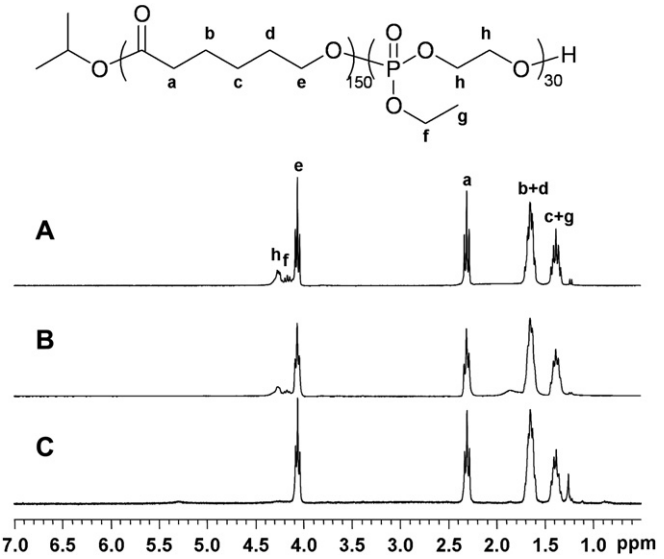


Fig. 4. ^1H NMR spectra of degradation products of PCL_{150}-*b*-$PEEP_{30}$ with phosphodiesterase I for 0 day (A), 3 days (B) and 8 days (C).

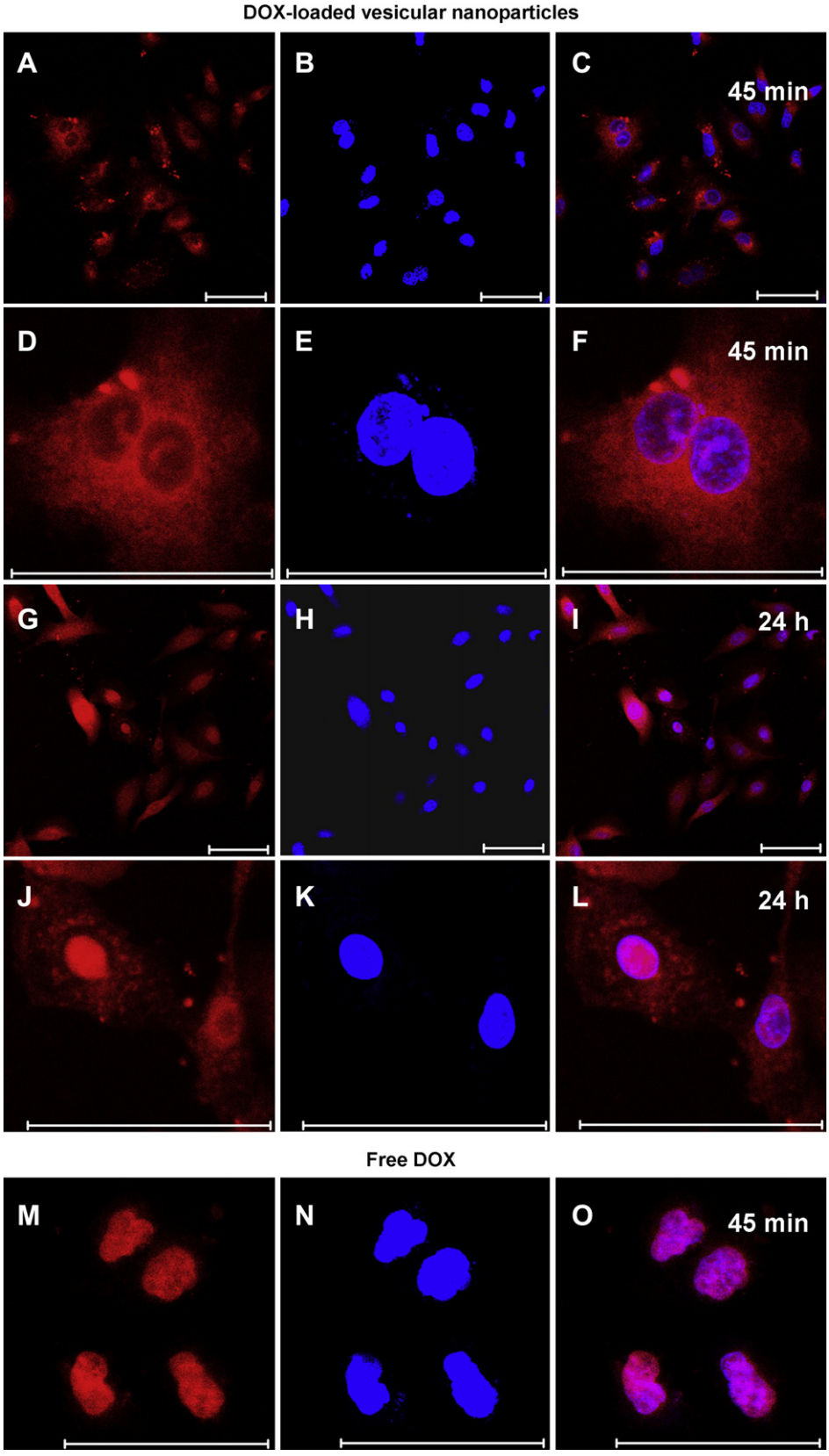


Fig. 5. CLSM images of A549 cells incubated with DOX-loaded vesicles for 45 min (A–F) and 24 h (G–L). M-O illustrates A549 cells incubated with DOX in free form for 45 min. The red fluorescence is from DOX (A, D, G, J, M) and the nuclei are stained with blue fluorescence dye Hoechst 33342 (B, E, H, K, N). The third column illustrates overlaid images while purple fluorescence indicates colocalization of DOX with Hoechst 33342. Scale is 50 μm in all images.

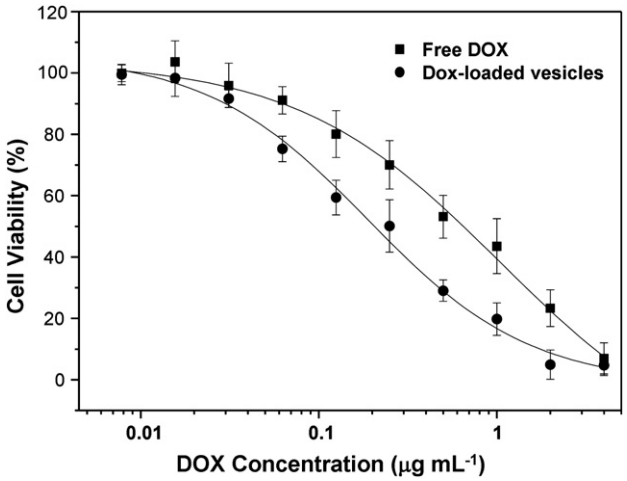


Fig. 6. *In vitro* inhibition to A549 cell proliferation in 72 h by DOX-loaded vesicles and DOX in free form at various doses.

PBS, or citric acid buffer with simultaneous sonication for at least 30 min. The suspension was then heated at 65 °C for at least 12 h. Typical optical image (in Milli-Q water) (data not shown) illustrates the empty round vesicular structure with a size from about 1 to 10 μm. Similar results were observed in PBS and citric acid buffer.

To more clearly visualize the vesicular structure, we imbedded PKH26 into the membrane of the vesicles. PKH26 is a fluorescence dye that can intercalate stably into hydrophobic environment. PKH26 was added to the vesicle solution and excess PKH26 was removed by dialysis. The vesicles were then subjected to observation under CLSM. Fig. 2A and B illustrate the continuous membrane structure of vesicles where hydrophobic PKH26 was intercalated.

It has been reported that many as-formed micron-sized polymer vesicles can be reduced in size by liposome-type extrusion techniques [4,42,43]. The as-formed vesicles of PCL_{150}-*b*-$PEEP_{30}$ via membrane hydration (diluted to approximately 0.25 mg mL^{-1} in Milli-Q water) were excluded through 100 nm polycarbonate membrane. After several passes through the membrane, reductions in vesicle diameter to values in close agreement to the membrane pore size were achieved. In Fig. 2C, TEM observation shows circular empty vesicular structure with an average diameter of about 100 nm, indicating that the vesicles of PCL_{150}-*b*-$PEEP_{30}$ are readily extruded. DLS analysis reveals that the extruded vesicles are with low polydispersity, showing a hydrodynamic diameter of 114 nm in average (Fig. 2D). The extruded vesicles were monitored for 6 weeks using DLS and were found to be generally stable since the average diameters did not significantly change.

The structure of vesicles allows the incorporation of both hydrophilic and hydrophobic drugs within the vesicle lumen and membrane respectively. To evaluate the potential of PCL_{150}-*b*-$PEEP_{30}$ vesicles in drug delivery, doxorubicin in hydrochloride salt form (DOX) was used as a model drug. DOX was entrapped into the vesicles using an acid gradient method as earlier reported [44]. Free DOX not encapsulated in the vesicles was removed by dialysis (MWCO 100 KDa) against Milli-Q water at 4 °C. The drug loading content and efficiency into the vesicles were estimated to be 4.38% and 43.8%, respectively, determined by HPLC analyses. The drug loading efficiency to vesicles can probably be affected by many factors, including the preparation method of vesicles (pH gradient), solvent, segments of block copolymers, and molecular weight of copolymer.

The release of DOX from PCL_{150}-*b*-$PEEP_{30}$ vesicles was carried out in PBS (pH 7.4, 0.01 M) with 5 mM Mg^{2+} at 37 °C, and monitored with HPLC measurements. Fig. 3 shows the cumulative release profiles of DOX from the vesicles with or without the catalysis of phosphodiesterase I at a final concentration of 5 Unit L^{-1}. The enzyme phosphodiesterase I is known to catalyze the degradation of polyphosphoesters [36] and it is present in cytosome or subcellular regions of human cells [45]. There was an immediate burst release of about 20% of loaded drug in the first 8 h in the absence of the enzyme. This may primarily due to the diffusion of DOX located close to the surface of vesicles. Release of DOX from the vesicles continued in the following 132 h and the accumulated release of DOX reached 34.5% of total encapsulated DOX without the addition of phosphodiesterase I. This should be a result of PCL matrix erosion and drug's intrinsic permeability through the vesicular membrane, which has also been observed from PEG-*b*-PCL vesicles [46]. However, incubation of the vesicles with phosphodiesterase I resulted in accelerated release. Up to 83.8% of total loaded amount was released in 140 h in the presence of the enzyme. To demonstrate such effect is due to the PEEP block degradation under phosphodiesterase I catalysis, we extracted the degradation product of vesicles using chloroform and analyzed their ^{1}H NMR. The results shown in Fig. 4 revealed that strength of signals assigned to protons of PEEP block reduced with increasing enzymatic incubation time. Wu C et al. reported that enzyme catalyzed degradation of micelles involved two steps including the adsorption of enzymes onto the particles and enzymatic hydrolysis of polymer chains [47]. Degradation of PCL_{150}-*b*-$PEEP_{30}$ vesicles catalyzed by phosphodiesterase I is possibly in a similar one-by-one fashion, which would result in collapsed or broken vesicles with complete release of encapsulated drug molecules.

To evaluate the potential of PCL_{150}-*b*-$PEEP_{30}$ vesicles for intracellular drug delivery, we incubated DOX-loaded vesicles with A549 tumor cells and observed the cells under CLSM at 45 min and 24 h. Confocal laser microscope images shown in Fig. 5 illustrate A549 cells incubated with DOX-loaded vesicles. At 45 min, it shows that the DOX fluorescence appeared in all the cells, residing mainly in cytoplasm (Fig. 5A–F). Minimal DOX was found in the nuclei at 45 min, which should be attributed to DOX release from the vesicles. This is unlike cells incubated with DOX in free form for 45 min, only showing DOX accumulation in the nuclei that was judged from the merged confocal images (Fig. 5M–O). With extended incubation of A549 cells with DOX-loaded vesicles to 24 h, it is obvious that DOX accumulates in the nuclei (Fig. 5G–L), most likely owning to the diffusion of released DOX from the vesicles into the nuclei. The delayed fluorescence appearance/accumulation of DOX from cytoplasm to cell nuclei infers that DOX-loaded vesicles were internalized into the cells and released the encapsulated DOX intracellularly.

The ability of DOX-loaded vesicles to inhibit proliferation of A549 cells was investigated using an MTT viability assay, and it is compared with DOX in free form. The A549 cells were treated with DOX-loaded vesicles at different DOX doses from 0.8×10^{-3} to 4 μg mL^{-1}. As shown in Fig. 6, DOX-loaded vesicles exhibited higher inhibition to A549 cell proliferation after 3 days culture in comparison with DOX in free form. The DOX dose required for 50% cellular growth inhibition (IC_{50}) is 0.21 μg mL^{-1} in treatment with DOX-loaded vesicles, which is only ~1/3 of that required for treatment with DOX in free form. This is possibly attributed to the persistently intracellular release of DOX from the vesicles. Free DOX is known to cause high systemic toxicity when it is administrated to animals [48]. Enhanced inhibition of DOX-loaded vesicles to tumor cell growth would be advantageous in lowering the dose of DOX in applications. On the other hand, nanoparticles can potentially accumulate in tumor tissue through enhanced permeation and retention effect, which would further minimize the systemic toxicity and improve the efficacy in cancer treatment [49].

4. Conclusions

We have demonstrated that well-characterized block copolymer PCL_{150}-*b*-$PEEP_{30}$ can form biodegradable vesicles in aqueous solution. The vesicles can be prepared using a thin-film hydration method. Exclusion of the as-formed vesicles through the membrane results in reduction of vesicle diameter to values in close agreement to the membrane pore size. DOX can be loaded into the vesicles using an acid gradient method, while the release of DOX from the vesicles is accelerated in the presence of an enzyme phosphodiesterase I, due to the catalyzed degradation of PEEP block. The DOX-loaded vesicles can be successfully internalized by A549 cells, which results in enhanced inhibition to A549 cell proliferation as compared with DOX in free form, owning to the sustained release of DOX intracellularly. Since nanoparticles can potentially accumulate in tumor tissue through enhanced permeation and retention effect, with the properties described above, vesicles based on the block copolymer of PCL and PEEP is attractive as drug carrier for cancer therapy.

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