

# Characterization of a triblock copolymer, poly(ethylene glycol)-polylactide-poly(ethylene glycol), with different structures for anticancer drug delivery applications

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**Abstract** Formulation optimization from the early steps plays an important role in the success of drug delivery system development. To optimize nano-sized micelles based on poly(ethylene glycol)-*b*-poly(lactide)-*b*-poly(ethylene glycol) (PEG-PLA-PEG) triblock copolymers, the effect of PLA composition on the physicochemical properties of micelles was studied. Doxorubicin (DOX) was used to be encapsulated into micelles. In vitro studies on the safety of triblock copolymers and cytotoxicity of DOX-loaded micelles compared to free DOX were done, using MDA-MB-231 cells. Critical micelle concentration and micelle size were found to be linearly dependent on the PLA molecular weight (MW). In addition, it was shown that long PLA-containing micelles had low stability. The sizes of DOX-loaded micelles were bigger than those of empty micelles. The loading amount of DOX into micelles as well as the release rate of DOX from micelles depended on the PLA MW. Triblock copolymers themselves did not show any toxicity over a wide range of concentration. DOX-loaded micelles killed more tumor cells than free DOX. In summary, difference in hydrophobicity can be a critical factor to determine the physicochemical properties of micelles composed of PEG-PLA-PEG, and thus, it can affect the drug delivery efficacy of micelles. After considering all related factors, PEG-PLA-PEG 2 kDa-6 kDa-2 kDa seemed to be the best polymer for further studies.

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**Keywords** Anticancer · Block copolymer · Drug delivery system · Nano · Micelle

## Introduction

Most anticancer drugs exhibit poor solubility in water, rendering the delivery of those agents quite challenging. The clinical application of anticancer drugs is also very limited due to high systemic toxicity. Attempts have been made in the field of drug delivery systems to overcome these obstacles and difficulties by controlling the structural composition of drug delivery systems [1, 2].

There has been great interest in the use of micelles based on amphiphilic block copolymers as drug delivery systems for anticancer therapeutics [3, 4]. In aqueous solution, amphiphilic block copolymers with the concentration higher than critical micelle concentration (CMC) self-assemble themselves forming core-shell structure with the hydrophobic core for the encapsulation of hydrophobic compounds [5]. Another attraction of block copolymers in the drug delivery application is that their chemical compositions can be easily controlled to obtain the appropriate properties in the formulated drug delivery systems [6–8]. A biocompatible water-soluble polymer, PEG, has widely been utilized as a hydrophilic block because of its excellent water solubility, chain mobility, nontoxic, and non-immunogenicity [9]. The hydrophobic blocks have been tailor-made corresponding to the physical/chemical properties required for functional drug carriers [10]. The hydrophobic blocks with their different natures, molecular weights (MWs), and structures can enable the micellar drug carriers to be more effective for some drugs by changing the drug-polymer compatibility, formation of a rigid core, and controlled drug release.

Poly(lactide) (PLA) is a biocompatible, biodegradable polymer with low immunogenicity and good mechanical properties that facilitate its use in pharmaceutical and biomedical applications, such as sutures, implants for bone fixation, drug delivery vehicles, and tissue engineering scaffolds [11–13]. Amphiphilic block copolymers composed of PLA and PEG, including diblock copolymer and triblock copolymer (ABA and BAB with PLA as A and PEG as B), have been extensively investigated as materials for drug delivery systems [14–17]. Among them, the micelles composed of BAB triblock copolymers seem to be the ideal drug delivery platforms, because they have a high PEG density on the surface, small sizes, and an enhanced stability [17–20]. We developed a BAB type, PEG-PLA-PEG triblock copolymer using Steglich esterification as a new synthesis method, which showed favorable properties, such as high stability, high drug loading efficiency, greater reconstitution property, etc., compared to PEG-PLA diblock copolymer in the previous reports [21]. This method could prepare the triblock copolymer without toxic linkers but with the functional groups at the end of polymer which could be decorated by imaging probes or targeting moieties [21]. To date, most of studies on nano-sized drug delivery systems have not focused on the long-term stability as well as on the formulation optimization for the best efficacy. The relationship between polymeric materials and drug delivery system properties as well final efficacy has

not been seriously taken into account. Since formulation optimization from the early steps can result in more opportunities to acquire the success in clinical trial and the huge financial saving, this investigation should be conducted carefully before any further studies.

The purpose of this study is to investigate the impact of PLA composition on the physicochemical characterizations of PEG-PLA-PEG triblock copolymers, micelles, and the *in vitro* anticancer efficacy of doxorubicin (DOX)-loaded micelles. A series of PEG-PLA-PEG triblock copolymers with the same PEG MW and different PLA MWs were synthesized and characterized as the platform for micellar systems for DOX, a typical anticancer drug. The relationships between the copolymer composition and micelle properties, including sizes, stability, and morphology, were investigated. DOX release profiles from micelles depending on the PLA block length were also evaluated. The toxicity of block copolymers themselves as well as DOX-loaded micelles against cancer cells was also investigated. The optimized formulation will be selected for further studies.

## Materials and methods

### Materials

Methoxy poly(ethylene glycol) (PEG, MW 2 kDa abbreviated as 2 K), L-lactide ((3S)-cis-3,6-dimethyl-1,4-dioxane-2,5-dione), *N,N*-dicyclohexylcarbodiimide (DCC), stannous octoate (Tin(II)-2-ethylhexanoate, Sn(Oct)<sub>2</sub>), 4-dimethylaminopyridine (DMAP), dimethylsulfoxide (DMSO), succinic anhydride, pyridine, and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF), toluene, acetone, and dichloromethane (DCM) were purchased from Honeywell Burdick & Jackson<sup>®</sup> (Muskegon, MI, USA). Doxorubicin (DOX)·HCl was purchased from Boryung Co. (Seoul, South Korea). Diethyl ether and hexane were purchased from Samchun chemical (Seoul, South Korea). MDA-MB-231 cells were obtained from Korean Cell Line Bank (Seoul, South Korea). DMEM medium, DPBS, penicillin–streptomycin solution, trypsin–EDTA solution, and fetal bovine serum (FBS) were purchased from Welgene (South Korea). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (USA).

### Methods

#### *Triblock copolymer synthesis*

PEG-PLA-PEG triblock copolymers were synthesized with the same procedures as described previously [21, 22]. First, PEG-PLA diblock copolymers were synthesized by ring-opening polymerization of L-lactide in the presence of PEG as initiator at 120 °C for 24 h, using Sn(Oct)<sub>2</sub> as catalyst and toluene as a solvent. PEG-PLA diblock copolymers synthesized by this method were precipitated by pouring the reaction mixtures into excess amount of cold diethyl ether. The precipitates were then filtered and dried in vacuum for 2 days. Carboxylated PEG (PEG-COOH) was

achieved by the reaction of PEG with succinic anhydride in the presence of DMAP, TEA, and pyridine. PEG, succinic anhydride, and DMAP were dissolved in 20 mL of DCM by stirring for 30 min. TEA and pyridine were then added. The reaction was carried out overnight, and the final products were obtained by precipitation in excess amount of diethyl ether, subsequent filtration, and drying in vacuum for 2 days. For PEG-PLA-PEG triblock copolymer preparation, the Steglich esterification was applied using the synthesized PEG-PLA and PEG-COOH. Both PEG-PLA and PEG-COOH were dissolved in DCM for 30 min with constant stirring. DCC as a coupling reagent and DMAP as a catalyst were added into DCM solutions. The reaction was carried out overnight, and the final products were obtained by precipitation in excess amount of diethyl ether, subsequent filtration, and drying in vacuum for 2 days.

### *Characterizations of block copolymers*

$^1\text{H-NMR}$  spectroscopy and gel permeation chromatography (GPC) were used to determine the MW and the composition of the block copolymers.  $^1\text{H-NMR}$  was performed using Varian, Gemini 2000 (NMR 300 MHz) instrument (Varian, USA).  $\text{CDCl}_3$  was used as a solvent for the analysis of block copolymers. The MW of the PLA segment was determined from  $^1\text{H-NMR}$  spectrum by examining the peak intensity ratio of the methyne proton of the PLA segment ( $\text{COCH}(\text{CH}_3)\text{O}$ :  $\delta = 5.2$  ppm) and the methylene protons of the PEG segment ( $\text{OCH}_2\text{CH}_2$ :  $\delta = 3.6$  ppm) based on the number-average MW of PEG [23]. Number- and weight-average MWs ( $M_n$  and  $M_w$ , respectively) as well as polydispersity index ( $M_w/M_n$ , PDI) of the copolymers were determined by GPC using Agilent Technology series-1200 instrument, equipped with the refractive index detector. THF was used as the mobile phase at 1.0 mL/min of flow rate. Column temperature was set at 30 °C. The copolymers were dissolved in THF, filtered, and injected into PLgel 10  $\mu\text{m}$  MIXED-B column (Agilent, USA). Block copolymer Mws were calculated based on the calibration curve made from a series of polystyrene standards (Scientific Polymer Products Inc., Ontario, NY) [14].

### *Preparation of self-assembled polymeric micelles*

Polymeric micelles were prepared by the dialysis method. PEG-PLA-PEG triblock copolymer (10 mg) was dissolved in 3 mL of DMSO. Triblock copolymer solution in DMSO was then transferred to dialysis membrane (MWCO, 3.5 kDa) (Spectrum, USA), and dialysis was carried out against 500 mL of phosphate buffer saline (PBS) pH 7.4 for 24 h. PBS pH 7.4 was replaced every 3 h.

### **CMC determination**

The fluorescent measurement for CMC determination was performed using a Scinco FS-2 fluorescence Spectrometer (Seoul, Korea). Fluorescence spectrometer was equipped with polarizers for excitation and emission of light beams. Pyrene was used as the fluorescent probe. The sample solutions were prepared by adding or rinsing pyrene solution in acetone to empty vials. After evaporating acetone, micelle

solutions with different concentrations of triblock copolymers were added to the vials to get final pyrene concentration of  $6 \times 10^{-7}$  M. These samples were stirred overnight at room temperature. Excitation spectra of pyrene in samples were recorded at  $\lambda_{\text{ex}} = 374$  nm at room temperature. CMC was estimated by plotting the ratio of  $I_1$  (intensity of peak at 336 nm) to  $I_3$  (intensity of peak at 334 nm) of the excitation spectra against the logarithms of the copolymer concentration. CMC was defined as the crossover point of low copolymer concentrations on this plot [24, 25].

### *Particle size measurement*

The sizes (effective hydrodynamic diameters) of micelles were measured by photon correlation spectroscopy using Zetasizer Nano-ZS (Malvern Instruments, UK) equipped with the Multi Angle Sizing Option (BI-MAS). The measurements were performed in a thermostatic cell at a scattering angle of  $90^\circ$ . Software provided by the manufacturer was used to calculate effective hydrodynamic diameter values.

### *Morphology observation*

The morphologies and sizes of the polymeric micelles were examined using field emission scanning electron microscopy (FE-SEM) (Hitachi-4800, Japan). A few drops of diluted micelle solutions were deposited onto a slide glass and dried. FE-SEM examinations were performed with platinum (Pt) coating on samples.

## **Preparation and characterizations of DOX-loaded micelles**

DOX·HCl was dissolved in DMSO to get the concentration of 2.5 mg/mL. TEA (molar ratio to DOX·HCl, 2:1) was then added to detach HCl from DOX. Triblock copolymer was dissolved in DMSO to get the concentration of 10 mg/mL. DOX solution (2 mL) was mixed with triblock copolymer solution (1 mL), and dialysis (MWCO, 3.5 kDa) (Spectrum, USA) was carried out against 500 mL of PBS pH 7.4 for 24 h. PBS pH 7.4 was replaced every 3 h. The sample was then collected and centrifuged at 5000 rpm for 5 min to precipitate un-trapped DOX. Supernatant which contained DOX-loaded micelles was then collected. Micelles were then broken down by diluting micelle solution with DMSO. The concentrations of DOX in micelles were determined by UV–VIS spectrometer (GENESYS 10 UV, Thermo Sci., USA) at wavelength  $\lambda = 481$  nm. The DOX loading capacity was calculated with the following equation:

$$\text{DOX loading capacity (wt/wt\%)} = (\text{DOX loaded in micelles/polymer and DOX}) \times 100.$$

## **DOX release from micelles**

For the drug release test, 1 mL of DOX-loaded micelle solutions was transferred into dialysis membrane tubes (Spectra/Por<sup>®</sup>, MWCO 3.5 kDa). The dialysis membrane tubes were subsequently immersed in a vial containing 10 mL of PBS pH 7.4 and

incubated in shaker water bath at a speed of 70 rpm and 37 °C. At predetermined time points (1, 3, 6, 9, 12, 24, and 48 h), the media in the vials were collected to determine the amount of DOX released and the vials were replenished with 10 mL of fresh PBS pH 7.4. The amount of DOX released from the micelles was quantified using UV–VIS spectrometer (GENESYS 10 UV, Thermo Sci., USA) at wavelength  $\lambda = 481$  nm.

### **In vitro cytotoxicity**

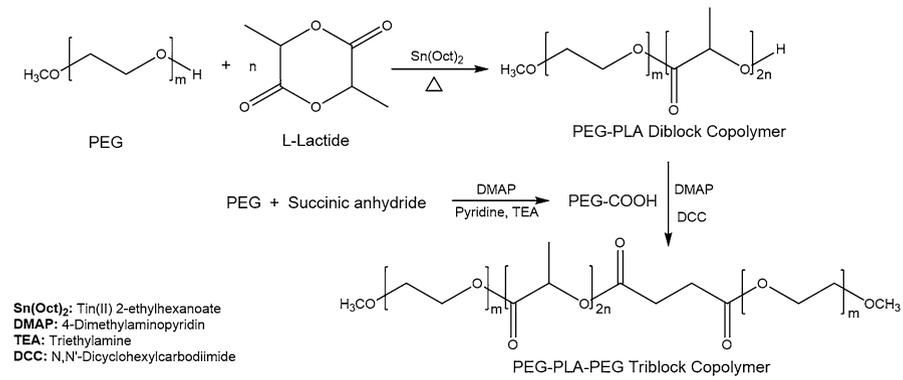
The cytotoxicity of triblock copolymers was assessed with CCK-8 viability assay against MDA-MB-231 cell line. The cells were seeded in 96-well plates at  $5 \times 10^3$  cells per well in 100  $\mu$ L of DMEM medium supplemented with 5 % FBS, 1 % penicillin–streptomycin, and incubated at 37 °C in 5 % CO<sub>2</sub> for 24 h. After that, the media were removed and 100  $\mu$ L of micelle solutions with different concentrations of triblock copolymers were added and incubated at 37 °C in 5 % CO<sub>2</sub> for 48 h. The fraction of living cells was determined by CCK-8 cell viability assay. The cytotoxicity of free DOX and DOX-loaded micelles was assessed with CCK-8 viability assay against MDA-MB-231 cell line. The cells were seeded in 96-well plates at  $5 \times 10^3$  cells per well in 100  $\mu$ L of DMEM supplemented with 5 % FBS, 1 % penicillin–streptomycin and incubated at 37 °C in 5 % CO<sub>2</sub> for 24 h. Then, the media were removed, and 100  $\mu$ L of free DOX solutions or DOX-loaded micelle solutions were added with different concentrations of DOX and incubated at 37 °C in 5 % CO<sub>2</sub> for 48 h. The fraction of living cells was determined by CCK-8 cell viability assay. IC<sub>50</sub> of free DOX and DOX-loaded micelles were calculated with the GraphPad Prism 5 software.

## **Results and discussion**

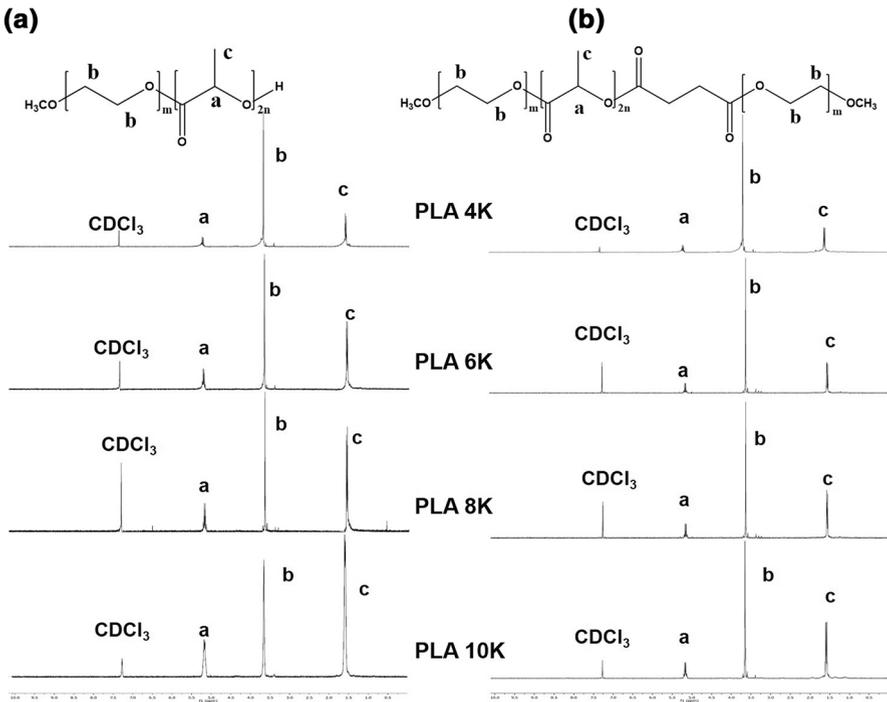
### **Triblock copolymer synthesis and characterizations**

A series of PEG-PLA-PEG triblock copolymers with the same MW of hydrophilic block (PEG 2 K) but different targeting MWs of PLA (4, 6, 8, and 10 kDa, abbreviated as 4, 6, 8, and 10 K, respectively) were synthesized by the Steglich esterification method between PEG-PLA diblock copolymers and PEG-COOH (Fig. 1) [22]. Succinic anhydride, a nontoxic material, was used as the link and the reaction was performed under minor condition (at room temperature) that negligibly affected the PLA backbone.

The success of triblock copolymer synthesis was confirmed by <sup>1</sup>H NMR (Fig. 2) and GPC (Table 1). The peak at 3.6 ppm was assigned to proton b of PEG. The peaks at 5.2 and 1.6 ppm were assigned to protons a and c, respectively, of PLA. There were obviously increased ratios of PEG/PLA proton peak intensities in triblock copolymers compared to those in diblock copolymers. Importantly, the MW of a block copolymer confirmed by <sup>1</sup>H NMR was consistent with the one determined by GPC. Except sample T10, the PDIs of all triblock copolymers were relatively low, indicating that the narrow polymer molecular distributions played an important role in the homogeneous formation of micelles.



**Fig. 1** Di- and triblock copolymer syntheses



**Fig. 2** <sup>1</sup>H NMR spectrum of **a** di- and **b** triblock copolymers. CDCl<sub>3</sub> was used as a solvent

### CMC determination

CMC defines the thermodynamic stability of the micelles. It is a key player in stabilizing micelles during the systemic circulation post injection [8, 26–28]. There exist only single chains of block copolymers below CMC in an aqueous environment, while both micelles and single chains of block copolymers co-exist

**Table 1** Characterizations of di- and triblock copolymers based on PEG 2 K

Targeting MW of PLA (Da)	Diblock copolymer			Triblock copolymer				
	$M_n$		PDI <sup>b</sup>	$M_n$		PDI <sup>b</sup>	Final structure <sup>c</sup>	Code
	<sup>1</sup> H NMR	GPC <sup>a</sup>		<sup>1</sup> H NMR	GPC <sup>a</sup>			
4 K	5400	5200	1.15	6600	7400	1.24	2 K–3.4 K–2 K	T4
6 K	7900	7800	1.29	9100	9700	1.31	2 K–5.7 K–2 K	T6
8 K	10,700	9300	1.22	12,200	11,100	1.30	2 K–7.1 K–2 K	T8
10 K	11,300	12,100	1.54	13,300	15,000	1.59	2 K–11.0 K–2 K	T10

$M_n$  number-average MW

<sup>a</sup> Based on polystyrene standards

<sup>b</sup> Polydispersity index (calculated from GPC data)

<sup>c</sup> Based on GPC data of triblock copolymers

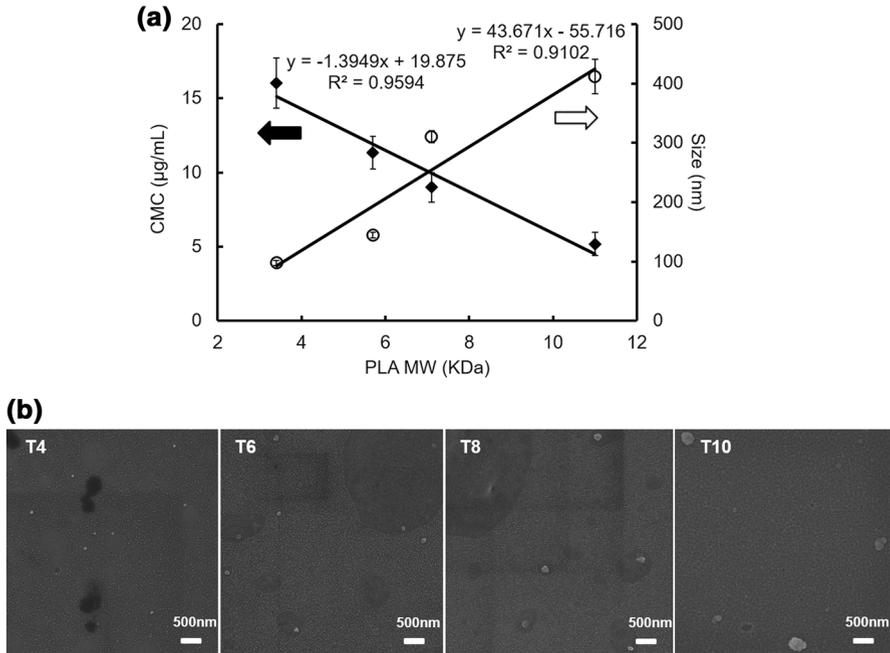
above it. Thus, micelles based on block copolymers with low CMC will probably have a high chance to reach the target site without dissociating into single chains and releasing the loaded drug.

In this study, the fluorescence spectroscopy method was employed for the CMC measurement using pyrene which is highly hydrophobic and preferentially migrates into the hydrophobic core of micelles in aqueous solutions as fluorescent probe [29]. Pyrene shows weak fluorescent intensity in polar environment (e.g., aqueous solutions), while it shows strong fluorescent intensity in non-polar environment, inducing a consequent sharp increase in the ratio  $I_1/I_3$  at CMC which can determine the CMC of triblock copolymers [29, 30].

The CMC of block copolymers with similar structures is dependent on the hydrophilic/hydrophobic ratio and inversely proportional to the hydrophobic ratio [8, 16]. From the results of our study, the inverse co-relation between CMC and hydrophobic ratio in triblock copolymers was confirmed (Fig. 3a, left column). Interestingly, a linear correlation between CMC and PLA MW in triblock copolymers was observed ( $R^2 = 0.959$ ). As mentioned above, the thermodynamic stability of polymeric micelles is strongly affected by the CMC of block copolymers and the copolymers with low CMC may increase the thermodynamic stability of micelles. Thus, triblock copolymers with long PLA seemed to be appropriate for developing drug delivery systems, because the copolymers with longer PLA had lower CMC which can enhance its thermodynamic stability. However, the development of a drug delivery system depends not only on the thermodynamic stability but also on other factors, such as simplicity of preparation, physical and chemical stability, drug loading capacity, etc. Thus, all those factors should be considered when choosing the best triblock copolymer for further investigation.

### Characterizations of micelles

Micelles based on PEG-PLA-PEG triblock copolymers with different lengths of PLA were prepared by the dialysis method, and the stability of micelles was monitored by determining particle sizes within a week post preparation.



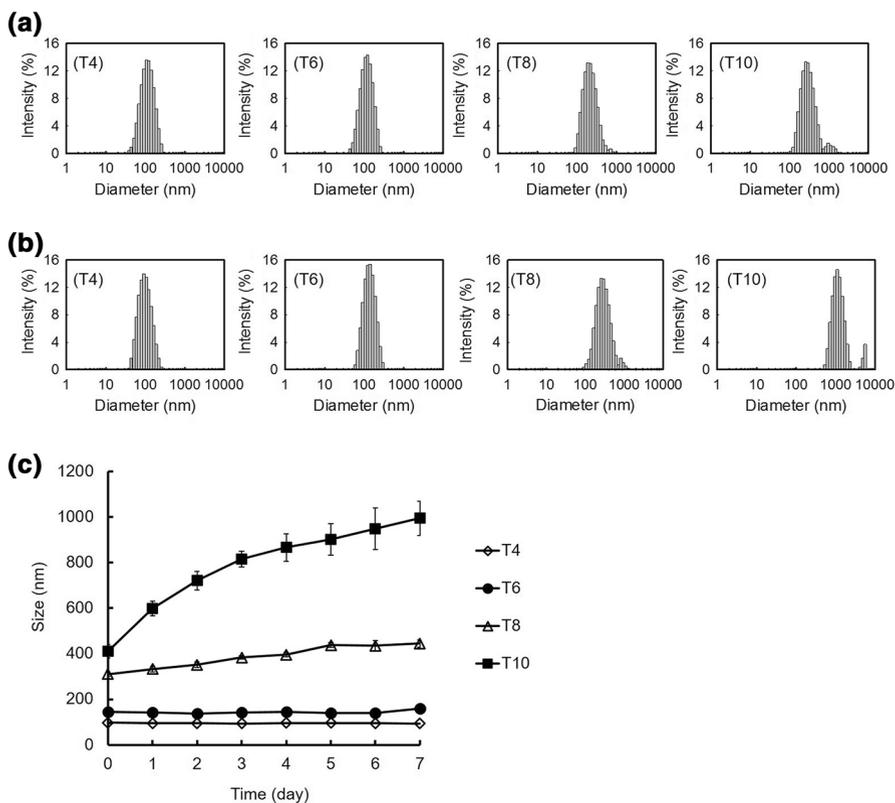
**Fig. 3** a Dependence of CMC (left column) and micelle size (right column) on PLA MW. b Morphology of micelles from different triblock copolymers

It was revealed that the micelle sizes increased with the increase in PLA MW (Fig. 3a, right column). T4 and T6 formed the micelles with sizes less than 200 nm, while the sizes of micelles prepared from T8 and T10 were higher than 300 and 400 nm, respectively. It was possibly due to the bigger micelle cores formed by higher MWs of PLA [16]. Interestingly, there was also linear correlation between PLA MW and micelle size ( $R^2 = 0.910$ ). This relation could be used to estimate the sizes of micelles formed from PEG-PLA-PEG triblock copolymers with different PLA MWs. Micelle sizes determined by FE-SEM were also consistent with those determined by Zetasizer (Fig. 3b). From the FE-SEM images of particles, an increase in the micelle sizes was observed when the MW of PLA increased. Despite the difference in the sizes, all polymeric micelles showed the spherical morphology. Except the case of T10, micelles from T4, T6, and T8 had unimodal and relatively narrow particle size distributions (Fig. 4a). The wide distribution of T10 micelle sizes was probably due to the high PDI of T10 polymer. Since the homogeneity is quite important for the development of drug delivery systems, T4, T6, and T8 micelles could be promising candidates for further studies.

To determine micelle's stability, micelles were stored at room temperature and their sizes were measured every day within 1 week after preparation (Fig. 4c). T10 micelle particle size increased as double at day 3 and it even further reached 1000 nm after 1 week, proving its low stability. T8 micelles also showed relatively limited stability with an increase in particle size within 1 week. On the other hand, T4 and T6

micelles had high stability with no change in particle size within 1 week. The PEG shell plays a crucial role in stabilizing micelles by generating steric repulsive forces which will compete with the inter-particle's Van der Waals attractive forces and the extent to which the PEG corona is able to sterically stabilize the micelles depends on both the surface density of PEG and the thickness of the PEG [8]. Due to the big difference in the hydrophobic and hydrophilic block length, the density of PEG on the surface of T10 micelles was not able to generate enough steric stabilization, resulting in micelle aggregations and bigger particle size. The histograms of micelle sizes also presented the stability of different systems. While the histograms of T4 and T6 micelles remained at the same positions around 100 nm after storing at room temperature for 1 week, those of T8 and T10 micelles shifted to the right, indicating an increase in micelle particle sizes (Fig. 4a, b).

From the results of CMC, micelle particle sizes, and stability of micelles according to hydrophobic block length, it indicated that the hydrophobic length of PEG-PLA-PEG triblock copolymers needed to be optimized in order for micellar systems to meet the requirement of thermodynamic stability as well as storage stability.



**Fig. 4** Size histograms of micelles determined **a** right after preparation and **b** after stored at room temperature for 1 week. **c** Size changing of micelles from different triblock copolymers within 1 week after preparation

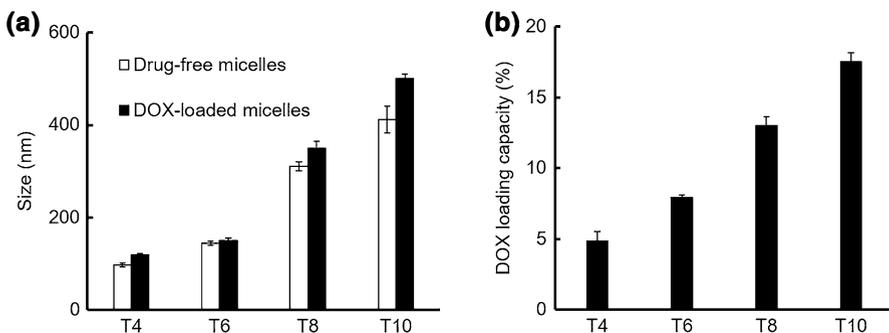
## DOX-loaded micelles based on triblock copolymers

Doxorubicin, a common anticancer drug, was chosen as a typical poorly water-soluble drug for loading into micelles based on PEG-PLA-PEG triblock copolymers, using the dialysis method. The DOX entrapped in the hydrophobic core caused an increase in the micelle particle sizes compared to drug-free micelles (Fig. 5a). DOX loading capacity of micelles increased with the increase of PLA MW (Fig. 5b) and it indicated that the physical entrapment of hydrophobic drug in polymeric micelles was triggered by the hydrophobic interaction between the drug molecule and the hydrophobic core of micelles [31]. Therefore, the higher MW of PLA provided more hydrophobic interaction between the drug molecule and the hydrophobic core for a better entrapment of DOX, resulting in enhanced loading capacity.

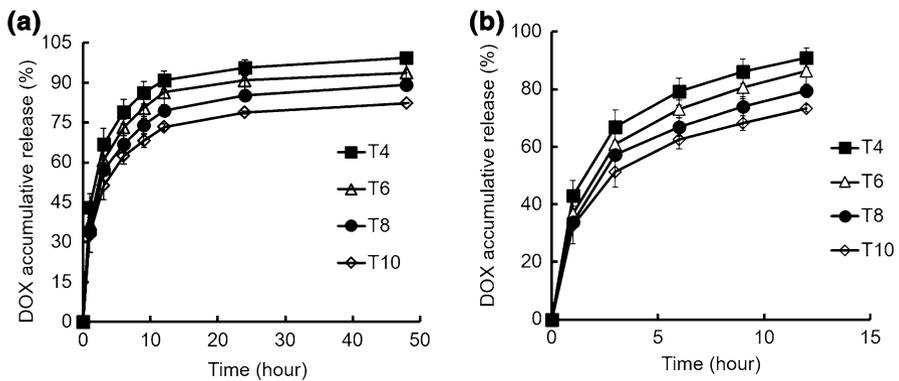
## DOX release from micelles

The release behaviors of DOX from micelles in PBS pH 7.4 were studied and represented in Fig. 6. For all micelle formulations, there was burst release effect in DOX release in the first hour, followed by a relatively sustained and slow release until 12 h. The rates of DOX released from micelles were affected by the composition of block copolymers. The rate of DOX release was inversely proportional to the PLA chain length. This difference was probably due to the fact that longer PLA tends to lower the diffusion rate of DOX from the micelle core. The difference in release rate of DOX from micelles may also be due to the degree of PLA crystallinity that is proportional to the length of PLA and the hydrophilic/hydrophobic ratio. Thus, longer PLA with higher degree of crystallinity caused a lower fluidity of the core [4], resulting in the slow diffusion of DOX.

The low stability of long PLA-containing micelles may also be a reason for low release rate of DOX due to the aggregation of those micelles during the process of release tests. These results were in accordance with the data on drug release from micelles based on PEG-PLA or PEG-PLGA [16, 32]. From this result, it is important to note that the DOX release rate from the micelles can be tuned by changing the hydrophilic/hydrophobic ratio of block copolymers.



**Fig. 5** Properties of DOX-loaded micelles. **a** Sizes of DOX-loaded micelles compared to those of drug-free micelles. **b** Loading capacity of DOX into different micelles

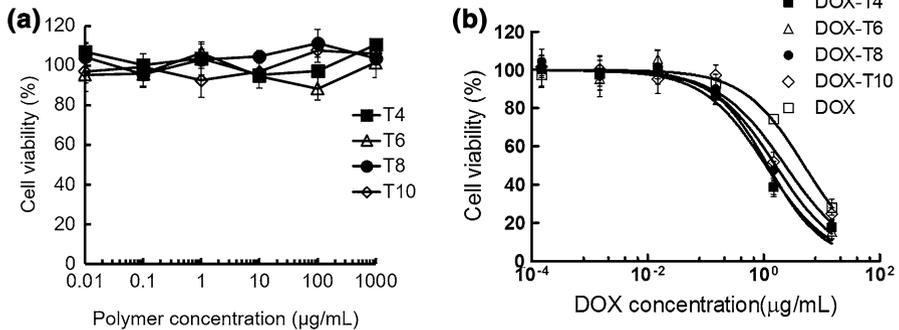


**Fig. 6** Release of DOX from micelles **a** in 48 h and **b** in the first 12 h

### In vitro cytotoxicity

The use of excipients is essential in drug formulations. Unfortunately, excipients may also cause serious adverse effects, such as the case of Taxol (Bristol-Myers Squibb Co.), which is a commercially marketed formulation of paclitaxel [33]. In this formulation, a relatively high concentration of cremophor is used to enhance the solubility of paclitaxel. However, since cremophor is not an inert excipient, it causes a wide range of biological effects, some of which have important clinical implications [34]. Thus, the use of Taxol is associated with severe hypersensitivity reactions [33]. Since the safety of excipients in drug formulations is critically important to avoid the adverse reactions, we determined the toxicity of synthesized triblock copolymers against MDA-MB-231 cancer cell line. It was revealed that all PEG-PLA-PEG triblock copolymers did not show any toxicity with the concentration up to 1 mg/mL (Fig. 7a). These toxicity data together with the DOX loading capacity suggested that our triblock copolymers could be very potential materials for increasing the solubility of poorly water-soluble drugs without remarkable complications related to toxicity.

The cytotoxicity of DOX-loaded micelles based on PEG-PLA-PEG triblock copolymers against MDA-MB-231 cancer cell line was estimated and compared with free DOX. As shown in Fig. 7b, both DOX-loaded micelles and free DOX clearly showed cancer cells growth inhibition. Among different DOX-loaded micelles, DOX-T4 and DOX-T6 micelles showed the highest anticancer effects, whereas DOX-T8 and DOX-T10 exhibited lower effects against MDA-MB-231 cell line. That can be attributed to the poor stability of T8 and T10 micelles and the slow releases of DOX from T8 and T10 micelles. More importantly, the DOX-loaded micelles showed significantly enhanced cytotoxicity with two- to fivefold decrease in IC<sub>50</sub> compared with free DOX. That might be due to the higher uptake of DOX from nanoparticles than from free DOX solution [10, 35]. These results demonstrated the potential applications of PEG-PLA-PEG triblock copolymers in designing delivery systems for anticancer drugs with the aim of improving anticancer efficacy.



**Fig. 7** Cytotoxicity of **a** triblock copolymers and **b** DOX-loaded micelles (compared with free DOX) against MDA-MB-231 cell line. The IC<sub>50</sub> values of DOX-T4, DOX-T6, DOX-T8, DOX-T10, and free DOX were 1.103, 1.190, 1.621, 2.369, and 5.022 µg/mL, respectively

## Conclusions

Biocompatible and biodegradable PEG-PLA-PEG triblock copolymers with the same hydrophilic block length (PEG 2 K) and different MWs of PLA were synthesized as platforms for the delivery of a poorly water-soluble anticancer drug, DOX. These amphiphilic block copolymers self-assembled into core-shell micelles, having PLA core that was capable of encapsulating DOX with relatively high loading capacities. The PLA length related to the hydrophobic/hydrophilic ratio can significantly affect the micelle sizes, stability, DOX loading capacity, release kinetic of DOX from micelles as well as cell cytotoxicity of DOX-loaded micelles. These block copolymers showed high safety with the concentration up to 1 mg/mL. After considering all of the aspects, such as stability, loading capacity, DOX-loaded micelle cytotoxicity, and T6 seemed to be the optimized PEG-PLA-PEG triblock copolymer. This polymer could be promising drug delivery systems for anticancer drugs.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

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