Destabilization of Liposomes by Uncharged Hydrophilic and Amphiphilic Polymers

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The destabilization effect of a hydrophilic polymer, poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA), and an amphiphilic polymer, PHEA-*g*-poly(2,2-dimethyltrimethylene carbonate) (PHEA-*g*-PDTC), on phosphatidylcholine vesicles has been investigated. The hydrophilic polymer PHEA has a strong destabilizing effect on the liposomes and induces immediate vesicular membrane leakage and aggregation, while the destabilizing effect of the amphiphilic polymer PHEA-*g*-PDTC is weaker. The in situ observation on shape transformations of a vesicle indicates that the addition of PHEA increases the amplitudes of fluctuations of the vesicle membrane, finally leading to the burst of the vesicle. The existence of hydrogen bonding between the polymers and the liposome membranes is mainly responsible for inducing the destabilization. The molecular structure of the polymers, such as the stiffness of the polymer backbone and the spacer connecting the hydroxyl group with the backbone, greatly affects the interaction between the polymers and the vesicles.

1. Introduction

Liposomes are microparticulate lipoidal vesicles that are extensively investigated as biomimetic membranes, due to their biomembrane-mimetic nature, and carriers for drug delivery and gene transfection, because of their unique structure allowing encapsulation of water-soluble compounds in the aqueous compartment and lipophilic materials in the lipid membrane.[1−3]

Interactions between polymers and liposomes are currently of substantial interest. Synthetic polymers and polypeptides have many different effects on lipid membranes.[4] Among them, the polymer-induced membrane fusion has attracted great attention, because the artificially induced cell fusion is an indispensable technology in the modern biotechnology, especially genetic engineering and cell technology.[5,6] For example, an important step in gene transfection is to transporting DNA across cell membranes. Other applications include inducing fusion between the cell from the source animal and the enucleated ovum in cloning technology and introducing signaling molecules or antibodies into targeted cells. The studies on membrane destabilization, partial destruction, and leakage of the inner compartment are expected to provide considerable knowledge in these immerging fields.

On the other hand, the liposome membrane strengthening and stabilization by polymers have also evoked a lot of interest. It is well-known that classical liposomal drug delivery systems administered by intravenous route are generally cleared rapidly from the circulation by the reticuloendothelial system (RES), which is definitely undesirable in drug delivery. The development of sterically stabilized long-circulating liposomes through surface modification by polymers represents a milestone in the research of liposomes. The design of lipid vesicles with a hydrophilic/steric polymeric barrier at their surface allows the modification of their release kinetics and reduces the uptake by the RES.[2,3,7−10]

Due to the importance of fundamental studies on the interactions between polymers and liposomes, increasing attention has been paid to this field. The polymers studied include uncharged polymers,[4−9,11,12] cationic polymers,[10,13−15] anionic polymers,[16,17] hydrophilic polymers,[4−6] and amphiphilic polymers.[7,8,11,12,14] The investigations were mainly focused on the effects of charges, hydrophilic and hydrophobic characters, molecular weight, and chain flexibility on the interactions. Taking account of electric charges, it was found that most commonly the uncharged hydrophilic polymers could form coating layers and stabilize the vesicles,[2,3,9] whereas charged cationic or anionic polymers have various and complicated effects on the vesicles. They may induce liposome membrane fusion,[13] leakage,[16] endocytosis,[18,19] or reduced permeability.[10,17] In view of hydrophilic and hydrophobic character, uncharged hydrophilic polymers tend to form hydrophilic coating layers on the surface of vesicles that prevent the aggregation of vesicles.[2,3] Block amphiphilic polymers may provide a better stabilizing effect on the vesicles by inserting their hydrophobic parts into the lipid layers as anchors and thus preventing the detachment of the polymers from the vesicles,[7,8] while graft amphiphilic polymers with hydrophobic side chains may induce vesicle aggregation, since the hydrophobic side chains from the same polymer backbone could be inserted into different vesicles.[12] In concern of the molecular weight, poly(ethylene glycol) (PEG) and its derivatives are extensively studied. For vesicle fusion, PEG with the molecular weight of 8000−10 000 is most effective in causing the aggregation and fusion of vesicles because of the depletion force. The lower molecular weight PEG does not have an enough size to generate a significant depletion force, while the higher molecular weight PEG adsorbs sufficiently on the vesicle surface, which results in the elimination the depletion force.[5,6] In the preparation of sterically stabilized long-circulating liposomes using hydrophobic modified PEG derivatives, most commonly the molecular weight of the PEG part is 2000. The PEG part with a higher molecular weight may cause difficulty for the hydrophobic part to anchor in the lipid layer,[3] while the PEG part with a lower molecular weight cannot provide an effective hydrophilic barrier. Upon considering the polymer chain flexibility, it is well

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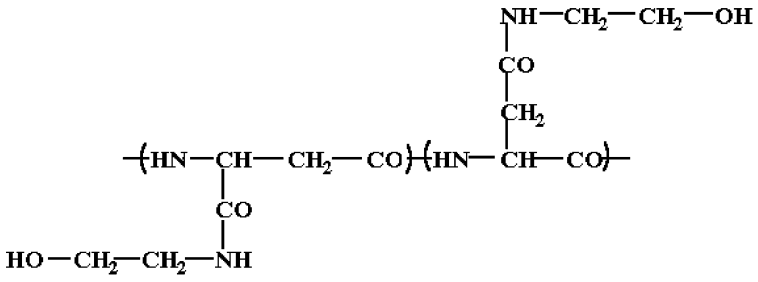
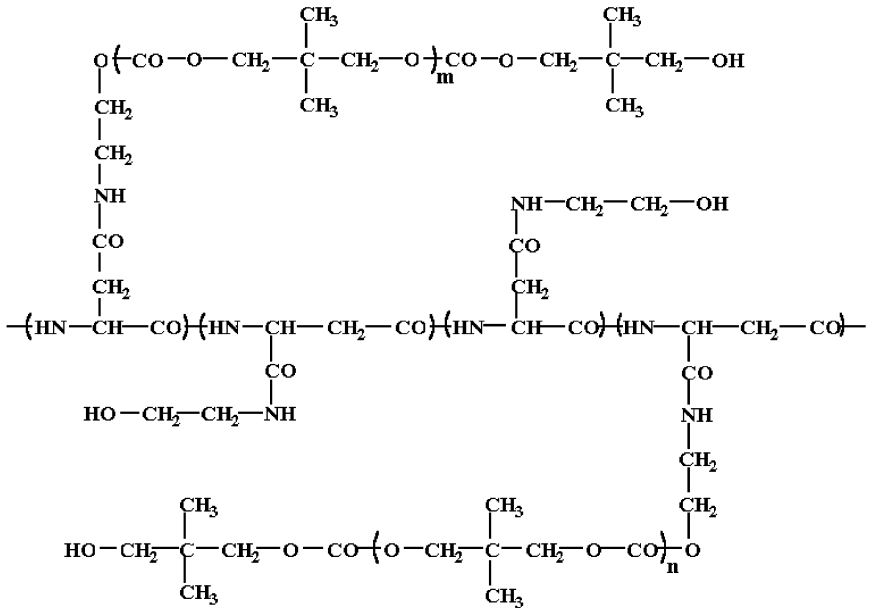
SCHEME 1. Chemical Formulas of PHEA and PHEA-*g*-PDTC



PHEA



PHEA-g-PDTC

accepted that the flexibility is important in imparting the long circulating property to the liposomes, because the flexible chains can form dense conformational "clouds" over the vesicle surface, while rigid polymer chains cannot form an effective protective layer on the membrane surface.[2]

Although a number of studies on the interaction between polymers and liposomes have been carried out, the exact role of polymers is still not clearly understood. The studies dealing with the effect of chemical structures on the polymer−liposome interactions are very limited, because a particular challenge to address this issue is to reveal the exact mechanism of these interactions and no unambiguous conclusion can be drawn from the present results. To clarify the mechanism of the polymer−liposome interaction, more information about the relationship between the chemical structure of polymers and the polymer−liposome interaction is required.

The purpose of our work is to study the effect of polymer structure on the polymer−liposome interaction. The destabilizing effect of two polymers, hydrophilic polymer PHEA and amphiphilic polymer PHEA-*g*-PDTC, on the liposome membranes has been investigated. The strong destabilizing effect of these polymers leads to the immediate damage of lipid vesicles. As far as we know, such a strong destabilization of an uncharged hydrophilic polymer with a relatively high molecular weight on vesicles has never been reported. From our experimental results, we found that the chemical structure of the polymer, such as the stiffness of the polymer backbone and the existence of a spacer in the side chain, strongly affected the interaction between the polymers and vesicles. These results doubtlessly provide useful information for the studies on the polymer−liposome interaction.

2. Experimental Section

2.1. Materials. *2.1.1. Materials for Polymer Synthesis.* 2,2-Dimethyl-1,3-propanediol (Shanghai Chemical Co., Shanghai, China) was dried in the vacuum oven at 60 °C for 2 h. Triethylamine was distilled over CaH_2. Ether chloroformate, L-aspartic acid, and phosphoric acid were of analytical grade and used as supplied by Shanghai Chemical Co. Ethanolamine (Shanghai Chemical Co.) was distilled before use. *N*,*N*-Dimethylformamide (DMF) was purified by distillation over P_2O_5 and CaH_2. Tetrahydrofuran (THF) was purified by distillation over sodium.

2.1.2. Lipids. Egg phosphatidylcholine (PC) and cholesterol (Chol) were purchased from Bio Life Science & Technology Co. Ltd. (Shanghai, China). Cholesterol was recrystallized from ethanol and dried in a vacuum oven before use.

2.1.3. Entrapped Drug. Tegafur (TFu) was purified from the Tegafur drug product of Jinan Pharmaceutical Factory. The details for purification are as follows. The drug pellets were ground and dissolved in ethanol. The starch in the drug pellets was removed by filtration. TFu was then recrystallized from ethanol−ether and dried in a vacuum oven.

2.2. Polymer Synthesis and Characterizations. *2.2.1. Molecular Design of Polymers.* Two polymers were prepared in this study. Their chemical formulas are shown in Scheme 1. The hydrophilic polymer poly-α,β-[*N*-(2-hydroxyethyl)-L-as-

partamide] (PHEA) is a water-soluble, nontoxic, nonantigenic biodegradable polymer with pending hydroxyl groups. The amphiphilic polymer PHEA-*g*-PDTC was synthesized by grafting hydrophobic biodegradable poly(2,2-dimethyltrimethylene carbonate) (PDTC) sequences onto the PHEA backbone.

To study the mechanism of the polymer−liposome interaction, we compared our polymers with poly(vinyl alcohol) (PVA). PVA (DP, 1750 ± 50; polydispersity, 1.50) was purchased from Shanghai Chemical Co. and used as supplied.

2.2.2. Synthesis of Hydrophilic Polymer PHEA. PHEA was synthesized by following a published procedure.[20] L-Aspartic acid (29.5 g) was mixed with phosphoric acid (15.0 g) in a 1 L round-bottomed flask. The flask was placed in a rotary evaporator and heated under reduced pressure in an oil bath at 180 °C for 2.5 h. The mixture was then dissolved in 200 mL of DMF and the solution added to water dropwise. The white precipitate that formed was filtered, washed with water until neutral, and dried in a vacuum oven at 110 °C for 24 h to obtain 19.7 g of poly-L-succinimide. The obtained poly-L-succinimide was dissolved in 200 mL of DMF and then added dropwise to a solution of 22.7 g of ethanolamine in 60 mL of DMF. The system was cooled in an ice bath. The mixture was stirred for 5 h and then poured into ethanol to precipitate the polymeric product. The product obtained was dissolved in DMF and the solution was poured into ethanol for precipitation again. The precipitate was then dissolved in water and dialyzed. The water solution was concentrated under vacuum and dried in a vacuum oven to obtain 24.0 g (Yield 71%) of PHEA.

2.2.3. Synthesis of Amphiphilic Polymer PHEA-g-PDTC. Monomer 5,5-dimethyl-1,3-dioxan-2-one (2,2-dimethyltrimethylene carbonate, DTC) was synthesized according to a literature procedure.[21] PHEA-*g*-PDTC polymer was synthesized by the ring-opening polymerization of DTC using PHEA with pendant hydroxyl groups as a macroinitiator without adding any catalyst.[22] DTC (0.451 g) and PHEA (0.549 g) were well-mixed and were placed in a dried glass flask with a magnetic stirring bar. The flask was evacuated, purged with argon three times, sealed, and then immersed in an oil bath that was preheated to 200 °C for 5 min. Then the reaction was allowed to proceed for 10 h at 120 °C. After the graft polymerizations, the product was dissolved in water and dialyzed for 48 h. Then the solution was concentrated under vacuum and the polymer was dried in a vacuum oven.

2.2.4. Polymer Characterizations. ^{1}H NMR spectra of polymers were recorded on a Mercury VX-300 spectrometer at 300 MHz.

Combined size-exclusion chromatography (SEC) and multiangle laser light scattering (MALLS) analysis was carried out to determine the molecular weight of the polymers. A dual detector system, consisting of a multiangle laser light scattering device and a differential refractometer, was used. The SEC system (Waters) was equipped with a G4000H8 column, when using THF as eluent, and TSK-GEL G5000 PWXL with G3000 PWXL columns, when using 0.2 mol/L NaCl water solution as eluent. The polymer concentration was 3 mg/mL and the flow rate was 1 mL/min. The MALLS (DAWN DSP, Wyatt Technology Co.) detector was operated at a laser wavelength of 632.8 nm.

2.3. Preparation of Liposomes. *2.3.1. Multilamellar Vesicles (MLVs).* The lipid mixture PC/Chol (4/1 mol/mol) was dissolved in chloroform, and the solvent was then evaporated, leaving a dry thin lipid film on the surface of the flask. After removal of the traces of organic solvents with nitrogen, the lipid film was hydrated with phosphate buffer solution with fast shaking by hand to form MLVs. The phosphate buffer solution (PBS) was prepared by adding 0.1 M NaH_2PO_4 (about 19 mL) to 0.1 M Na_2HPO_4 (about 81 mL) until the pH became 7.4.

2.3.2. Large Unilamellar Vesicles (LUVs). LUVs were prepared by the reverse-phase evaporation technique. The lipid mixture PC/Chol (4/1 mol/mol) was dissolved in chloroform and then dried in a rotary evaporator to form a thin film on the flask. The lipid film was redissolved in ether, and PBS was then rapidly added. The mixture was sonicated at 4 °C in a bath sonicator to obtain a homogeneous emulsion, and then the organic solvent was removed under vacuum at 30 °C. The resulting suspension was then extruded through a 0.4 μm pore diameter polycarbonate membrane filter several times to obtain the liposome suspension with a total lipid (PC and cholesterol) concentration of 4 mg/mL.

2.3.3. LUVs Encapsulating TFu. Vesicles encapsulating TFu were prepared by a method similar to that for the preparation of LUVs. A mixture of PC, cholesterol (PC/Chol 4/1 mol/mol), and TFu was dissolved in chloroform, and then dried in a rotary evaporator to form a thin film on the flask. The lipid film was redissolved in ether, and PBS was then rapidly added. The mixture was sonicated at 4 °C in a bath sonicator to obtain a homogeneous emulsion, and then the organic solvent was removed under vacuum at 30 °C. The resulting suspension was then extruded through a 0.4 μm pore diameter polycarbonate membrane filter several times to give LUVs encapsulating TFu. Gel filtration of the liposome suspension was then performed on Sephdex G 50 (Bio Life Science & Technology Co. Ltd.) to remove the unentrapped TFu. The encapsulation efficiency was 41%. The obtained liposome suspension had a lipid concentration of 4 mg/mL and an encapsulated TFu concentration of 0.164 mg/mL.

2.4. Polymer−Liposome Interaction Study and Liposome Characterizations. *2.4.1. Permeability Measurement.* To study the destabilizing effects of polymers on liposome membranes, the permeabilities of the liposomes without and in the presence of polymers were determined by the release of TFu. To prepare the polymer solutions, PHEA was dissolved in PBS to obtain polymer solutions with different concentrations of 30, 60, and 120 mg/mL, respectively. PHEA-*g*-PDTC was dissolved in PBS to obtain the polymer solution with a concentration of 60 mg/mL. To study the interaction between polymers and liposomes, 2 mL of polymer solution with a certain concentration was added into 6 mL of a suspension of LUVs encapsulating TFu to form a polymer−liposome mixture system with a lipid concentration of 3.0 mg/mL and an encapsulated TFu concentration of 0.123 mg/mL. For comparison, 2 mL of PBS without any polymer was added to 6 mL of suspension of LUVs encapsulating TFu to get the suspension with the same lipid and drug concentrations for carrying out further drug release measurement. PVA solution (2 mL) with a concentration of 60 mg/mL was also added into 6 mL of a suspension of LUVs encapsulating TFu to study the effect of PVA on LUVs.

To determine the release profiles of TFu, 8 mL of polymer−liposome mixture system was placed in a dialysis bag and then immerged in 50 mL of PBS. The dissolution tests were carried out in a shaking water bath (Grant OLS 200) at 37 °C. The drug concentration was determined by UV spectroscopy (Perkin-Elmer Lambda Bio 40 UV/VIS spectrometer) at 271 nm.

The dialysis membrane (MWCO 8000−12 000) allowed fast drug diffusion, and the free drug could be completely diffused out from the dialysis bag in a few hours. Since the release of drug from liposomes we prepared took a much longer time

TABLE 1: Properties of PHEA and PHEA-*g*-PDTC Polymers[22]

	^{1}H NMR[a]		SEC−MALLS[b]		
polymer	HEA:DTC in polymers (mol:mol)	−OH groups remaining (%)	M_w (g/mol)	M_w/M_n	R_g (nm)
PHEA		100	41 600	1.27	18.1
PHEA-*g*-PDTC	80:20	85	38 000	2.20	21.6

[a] In ^{1}H NMR characterization, D_2O was used as a solvent. [b] In SEC-MALLS analysis, H_2O was used as an eluent.

period of several days, the time-lag effect of dialysis membrane on the release rate could be ignored.

2.4.2. Morphology Observation. The morphologies of LUVs with and without the existence of PHEA were observed by transmission electron microscopy (TEM). TEM images were obtained by a JEM-100C transmission electron microscope. Negative staining with 2.0% phosphotungstic acid was performed to enhance image quality. To examine the effect of PHEA on LUVs, the PHEA solution was added into the suspension of LUVs to form a polymer−liposome mixture system with a lipid concentration of 3.0 mg/mL and a PHEA concentration of 10 mg/mL, and then the TEM visualization was carried out.

The interaction between PHEA and a lipid vesicle was in situ observed by polarizing light microscopy (PLM). The MLV suspension sandwiched between two glass slides was observed under an Olympus BX51 microscope, and a drop of PHEA solution was then added from the edge of glass slides to destabilize the MLVs. The optical images were recorded at particular time intervals by the software Linksys 2.43.

2.4.3. Measurement of Vesicle Size and ζ-Potential. The vesicle sizes and ζ-potentials of LUVs before and after adding polymers were measured by a Zetasizer 2000 (Malvern Instruments). To examine the effect of polymers on LUVs, the polymer solution was added into a liposome suspension to form a polymer−liposome mixture system with a lipid concentration of 3.0 mg/mL and a polymer concentration of 10 mg/mL.

3. Results and Discussion

3.1. Polymers in the Study on Polymer−Liposome Interaction. In this study, a hydrophilic polymer, PHEA, and an amphiphilic polymer, PHEA-*g*-PDTC, were identified to investigate the interactions between the polymers and lipid vesicles. By ^{1}H NMR and SEC characterization, the chemical structure and molecular weight of the polymers were determined as shown in Table 1. During the synthesis of PHEA-*g*-PDTC by grafting PDTC sequences to PHEA backbone, 15% of the hydroxyl groups in PHEA were replaced by PDTC sequences, because of the initiation of DTC polymerization by these hydroxyl groups, while 85% of the unreacted hydroxyl groups still remained in the resultant polymer. According to the ^{1}H NMR spectrum, we know that the average number of DTC units is 1.7 in each branch for PHEA-*g*-PDTC, which means that the hydrophobic branches are short.[22] That is the reason that PHEA-*g*-PDTC is still soluble in water.

To study the mechanism of the interaction between these polymers and liposomes, we compared our polymers with PVA, which has been extensively studied for polymer−liposome interaction and also has pending hydroxyl groups.

3.2. Interaction between Polymers and Vesicles. *3.2.1. Significant Increase in Liposome Permeability in the Presence of Polymers PHEA and PHEA-g-PDTC.* To study the destabilizing effects of polymers on liposome membranes, the permeabilities of the liposomes without adding polymers and after adding polymers were evaluated by monitoring the release profiles of TFu. TFu is an anticancer agent. It has been shown to be active in advanced or metastatic gastrointestinal cancer and in the adjuvant treatment of high-risk gastric cancer and has presented radio-enhancing effects in pilot human cancer trials.[23] In our study, TFu was used as an encapsulated drug to investigate the permeabilities of the liposomes. As shown in Figure 1, both release rates for the liposomes in the presence of PHEA and PHEA-*g*-PDTC are much faster than that of the liposomes without the presence of polymers. For example, after releasing for 45 h, 68% of the TFu has been released from the liposomes in the presence of PHEA and 50% of the TFu has been released in the presence of PHEA-*g*-PDTC, while only 20% of theTFu has been released without adding any polymer. In the presence of PVA, the TFu release rate becomes even slower than that of the liposomes without adding any polymer. This is in good agreement with previous studies.[2,8] According to the pervious literature, this is possibly due to the formation of a hydrophilic PVA layer on the surface of the lipid vesicle,[8] which hinders the release of TFu in vitro. From these release data, we can conclude that both hydrophilic PHEA and amphiphilic PHEA-*g*-PDTC increase the permeability of the liposome membrane. Compared with amphiphilic PHEA-*g*-PDTC, hydrophilic PHEA has a more obvious destabilizing effect.

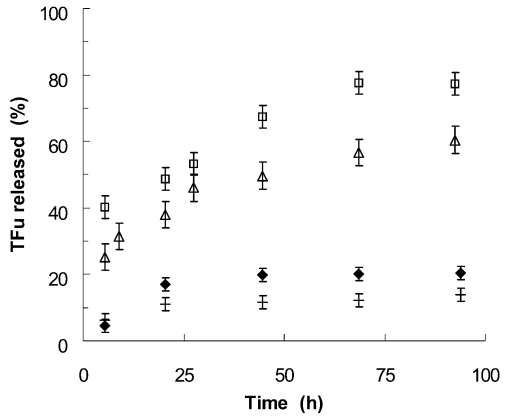
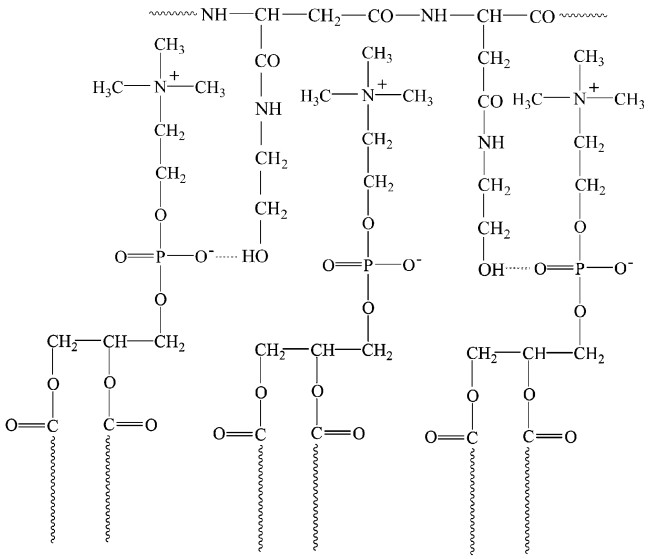


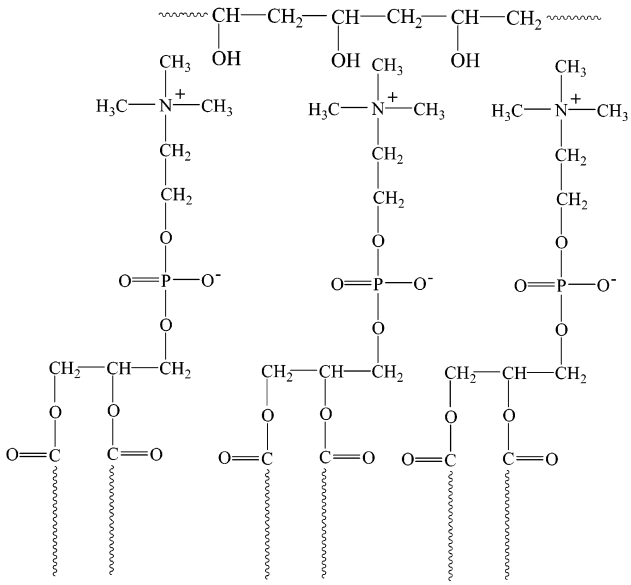
Figure 1. Release profiles of TFu from liposomes under different conditions: (◆) without the presence of polymers, (□) in the presence of PHEA, (△) in the presence of PHEA-*g*-PDTC, (+) in the presence of PVA. The lipid concentration was 3.0 mg/mL, the encapsulated TFu concentration was 0.123 mg/mL, and the polymer concentration was 15 mg/mL.

There are various interactions between polymers and lipid vesicles. For polymers and vesicles with specific physicochemical properties, the interactions may include depletion force, electrostatic force, hydrophobic interaction, hydrogen bonding, and so on.[4−6,24] For entropic reasons, the polymer chains avoid the space between two close particles; thus, the colloid particles are driven together, resulting from polymer depletion. The polymer-induced depletion interaction between vesicles is believed to be the main reason for the fusion of vesicles induced by hydrophilic polymers such as PEG.[5] According to previous studies of other researchers,[6] as the molecular weight increases, a crossover from depletion to adsorption occurs. At a certain critical chain length, a sufficient number of polymer segments can be absorbed on the vesicle surface to eliminate the depletion force. For PEG, this critical molecular weight is about 8000−10 000. In our study, both PHEA and PHEA-*g*-PDTC have relatively high molecular weights up to 41 600 and 38 000, respectively, which implies that the depletion forces in our

SCHEME 2. Hydrogen Bonding between PHEA and PC in the Liposome Membrane



SCHEME 3. Difficulty in Forming Hydrogen Bonding between PVA and PC in the Liposome Membrane



systems should not be the critical reason for destabilization. In our investigation, since both hydrophilic and amphiphilic polymers studied are uncharged neutral polymers, it is reasonable to assume there is no strong electrostatic interaction exists between the polymers and the vesicles.[4] It is well-known that amphiphilic polymers may interact with lipid vesicles by inserting their hydrophobic parts into the lipid membranes as a result of the hydrophobic interaction. The hydrophobic anchors usually induce the coating of the polymers on the vesicle surface, if the amphiphilic polymers are linear polymers, or the aggregation of vesicles, if the amphiphilic polymers are graft polymers with hydrophobic side chains.[7,8,12] For our polymer−liposome systems, based on the release profiles, the destabilizing effect of hydrophilic PHEA is stronger than that of amphiphilic PHEA-*g*-PDTC, which indicates that the hydrophobic interaction is not the major reason for inducing destabilization either. This experimental result is reasonable, because the hydrophobic interaction, most commonly, does not induce destabilization.[7,8,12] And in our system, the length of the hydrophobic branches of PHEA-*g*-PDTC is short, which may not be long enough to result in the effective anchor.

As we know, hydrogen bonding (H-bonding) is one of interactions responsible for inducing fusion.[4] On the basis of the previous discussion, we can deduce that the side chain with a hydroxyl group (−$CONHCH_2CH_2OH$ or −$CH_2CONHCH_2CH_2OH$) plays a critical role in inducing the destabilization of liposome membranes, because hydrogen bonds (H-bonds) can be formed between the −OH groups and the O atoms connected to P atoms in PC molecules. Except for the H-bonding, we indicate in Scheme 2 that there is no other strong interaction between PHEA and PC. Although −CONH− in PHEA and $-N^+(CH_3)_3$ in PC are close to each other, the hydrogen bonding cannot be formed between them, because N^+ is an electron-deficient site. And the attraction between the electron-deficient N^+ and the electron-rich O atom in −CO− is very weak, because of the steric hindrance caused by the three $-CH_3$ groups.

Although PVA has a similar chemical structure with hydroxyl groups as lateral groups, the interaction between PVA and liposomes is totally different from the interaction between PHEA or PHEA-*g*-PDTC and liposomes. To explain this phenomenon, we propose that the spacer between the −OH group and the polymer backbone for PHEA or PHEA-*g*-PDTC is essential for H-bond formation, as shown in Scheme 2. To form the H-bonds, the electron-deficient H atoms in the −OH groups in the polymers must access closely the O atoms with lone pairs connected to P atoms. Because the alignment of the PC molecules in the liposome membrane causes the $-N^+(CH_3)_3$ groups to be in the outside of the lipid membrane, only the H atoms that are connected to the polymer backbone through spacers can access the O atoms. This strongly implies that PHEA and PHEA-*g*-PDTC can form H-bonds with PC because their −OH groups are linked with the backbone through spacers $-CONHCH_2CH_2-$ or $-CH_2CONHCH_2CH_2-$. However, it is difficult to form H-bonds between PVA and PC, since PVA does not have spacers to connect −OH groups with its backbone and thus these −OH groups are not able to access O atoms (Scheme 3). In other words, for PHEA or PHEA-*g*-PDTC, the strong interactions (H-bonds) between polymers and vesicles destabilize the liposome membrane and finally lead to the membrane leakage. For PVA, there is no strong interaction existing between the polymer and the vesicles.

We also studied the effect of polymer concentration on the destabilization. Figure 2 shows the release profiles of liposomes in the presence of PHEA with different concentrations. Clearly, the release rate increases with increasing polymer concentration. The release from liposomes with the highest PHEA concentration of 30 mg/mL is fastest.

3.2.2. Leakage, Aggregation, and Deformation Induced by Polymer PHEA. The morphology of LUVs after adding polymer PHEA was observed by TEM. The TEM images are shown in Figure 3. The plain liposomes (Figure 3a) without adding PHEA are spherical particles. After adding PHEA, two kinds of morphological changes can be detected: one is leakage, and the other is aggregation. The time period between adding the polymer to the LUV suspension and getting the TEM images is about 10 min. Our TEM characterization shows that most of the vesicles have been damaged during this short time period. From Figure 3b1,2, we can find that, after adding the hydrophilic polymer PHEA, the size of the particles abruptly decreases and the shape of the particles becomes irregular, indicating the leakage of lipid vesicles and the release of the interior aqueous content. Figure 3b2 shows a typical image of aggregation of lipid particles after the damage of vesicles because of the addition of PHEA.

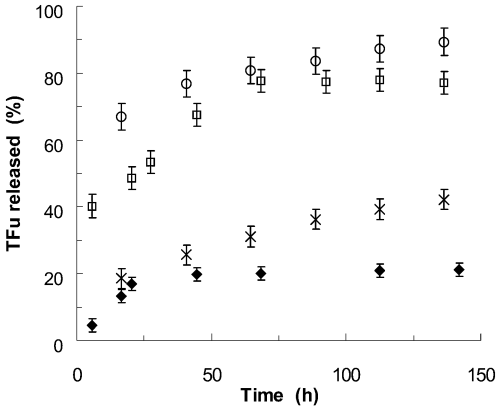


Figure 2. Release profiles of TFu from liposomes in the absence and presence of PHEA with different concentrations: (◆) without PHEA, (*) with PHEA at a concentration of 7.5 mg/mL, (□) with PHEA at a concentration of 15 mg/mL, (○) with PHEA at a concentration of 30 mg/mL. The lipid concentration was 3.0 mg/mL and the encapsulated TFu concentration was 0.123 mg/mL.

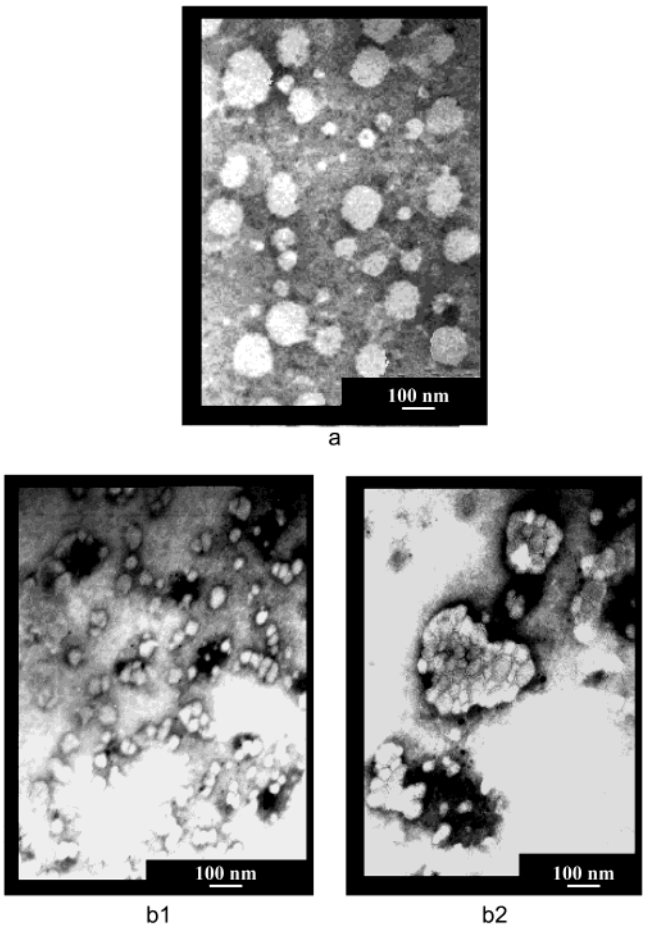


Figure 3. TEM images of (a) plain liposomes without adding polymer, (b1) liposomes after adding PHEA (leakage), and (b2) liposomes after adding PHEA (leakage and aggregation). No drug was loaded in the liposomes for TEM characterization.

To in situ study the morphology change after adding PHEA, we monitored the whole process of the shape change of a MLV after adding PHEA under a polarizing light microscope. As shown in Figure 4, the MLV has a spherical shape with a diameter of 12 μm before adding PHEA. After adding PHEA, continuous deformation of the MLV can be observed. The deformation finally induces the membrane leakage and damage of the vesicle within 1 min. The in situ observation of vesicle shape transformations indicates that the addition of PHEA results in the increased amplitudes of the fluctuations of the vesicle membrane, which leads to unstable excited states of the vesicle.

When a polymer is brought close to a membrane, the conformational fluctuation of the polymer chain is reduced due to the restriction of available space. The polymer is thus adsorbed on the membrane if the attractive interaction dominates the entropy reduction due to the space confinement, while it desorbs from the membrane surface if the entropy dominates over the interaction.[25] In our systems, the strong interaction (H-bonding) between the polymers (PHEA or PHEA-*g*-PDTC) and the PC leads to the adsorption of polymers onto the liposome membranes. The strong interaction between the polymer and the liposome membrane induces the membrane to bend toward the polymer to decrease the interaction energy. This deformation results in increased amplitudes of the fluctuations of the vesicle membrane, finally leading to bursting of the vesicle. This is in good agreement with literature results.[25]

As mentioned before, compared with PHEA, the attractive interaction between PVA and the liposome membrane is much weaker. For the weak-to-moderate adsorption of flexible polymer, the conformational entropy gain dominates over the cost of bending energy for free-energy minimization, leading to the positive curvature,[25] which is not able to induce the destabilization of the vesicle.

Another important aspect that needs to be emphasized is the chain stiffness. The hydrophilic polymer PHEA has a backbone with a certain rigidity because of the existence of amide linkages in the backbone. The flexibility of PHEA is much lower than that of some other hydrophilic polymers such as PVA, PEG, poly(acrylamide) (PAAm), and poly(*N*-vinyl-2-pyrrolidone) (PVPo), in which $-CH_2CH-$ and/or $-O-$ are presented in their backbones. Compared with flexible chains, the semiflexible chains have fewer opportunities to form favorable conformations coating the surface of the lipid vesicles. As a result, because of the existence of the strong interaction between the polymers and the liposome membrane, our semiflexible polymers give rise to the possibility of inducing membrane destabilization.

3.2.3. Changes in Particle Size Induced by Polymers. The particle size distributions of LUVs before and after adding polymers are presented in Figure 5. Before adding any polymer, the plain liposome has a mean size of 278 nm (Figure 5a). After adding PHEA, the mean size of particles immediately shifts to 1237 nm. A similar phenomenon can be detected after adding PHEA-*g*-PDTC. The mean size of particles increases to 994 nm. Obviously, the increase in particle size after adding polymer PHEA or PHEA-*g*-PDTC is due to the aggregation induced by liposome membrane leakage. The particle size data are in agreement with TEM observation. For comparison, we also measured the particle size after adding PVA. As expected, the mean size of particles after addition of PVA slightly increases. A possible reason for the slight increase in the vesicle size is the formation of a PVA layer over the surface of the liposomes. This is in accordance with the results reported in the pervious literature.[8]

3.2.4. Changes in ζ-Potential Induced by Polymers. The ζ-potentials of LUVs before and after adding polymer PHEA are shown in Table 2. The ζ-potential of plain liposome suspension is -9.6 at a pH of 7.4. PC is a zwitterionic lipid. Although a PC molecule is electrically neutral, the distribution of charges in its polar head region is asymmetrical. The negative ζ-potential of the plain liposomes is caused by the orientation of PC molecules in the liposome membrane. This observation is consistent with pervious studies.[26] After adding PHEA, the

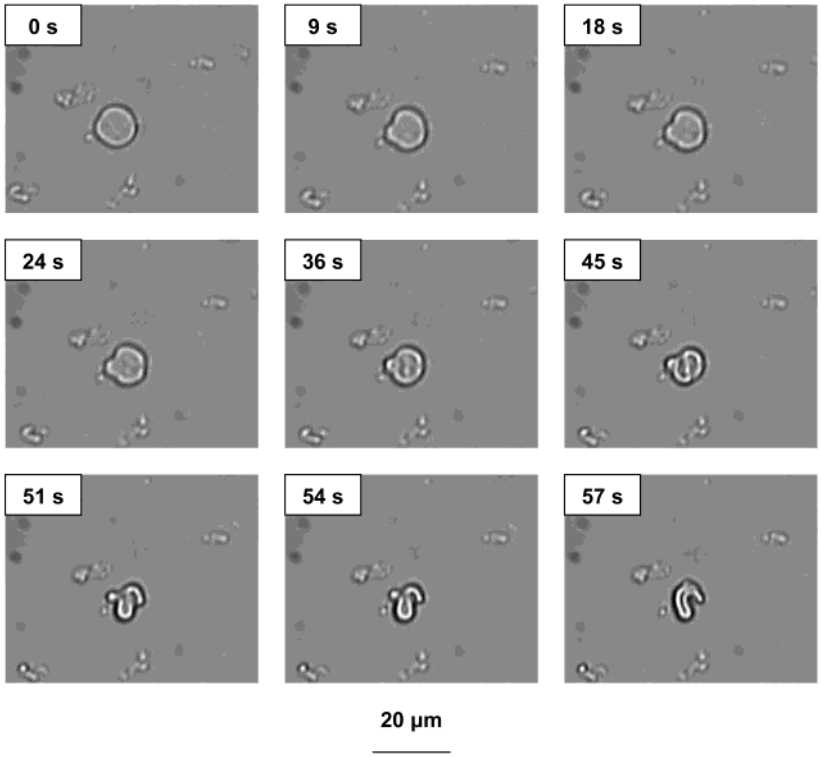


Figure 4. Deformation of liposome induced by PHEA. No drug was loaded in the liposomes for the deformation observation.

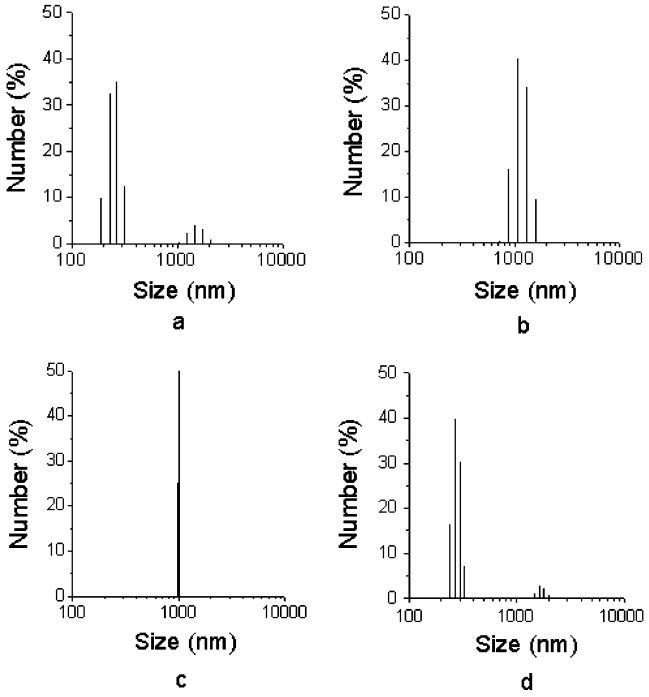


Figure 5. The size distribution patterns of liposomes in the absence and presence of polymers: (a) plain liposomes without polymer, (b) in the presence of PHEA at a concentration of 10 mg/mL, (c) in the presence of PHEA-*g*-PDTC at a concentration of 10 mg/mL, (d) in the presence of PVA at a concentration of 10 mg/mL. The lipid concentration was 3.0 mg/mL. No drug was loaded in the liposomes in the size measurements.

ζ-potential suddenly changes to 19.4. This is due to the fact that the addition of PHEA to the liposome suspension leads to the complete damage and aggregation of liposome membranes. We should note that the absolute value of the ζ-potential of the polymer−liposome mixture system is not very meaningful, because most of vesicles have already been damaged and the particles formed are actually the aggregates of damaged lipid membranes.

TABLE 2: ζ-Potentials of Liposomes with and without the Presence of Polymer

sample[a]	ζ-potential (mV)
plain liposome	−9.6
PHEA−liposome mixture system (PHEA:10 mg/mL)	19.4

[a] The lipid concentration was 3.0 mg/mL.

4. Conclusions

Hydrophilic polymer PHEA has a strong destabilizing effect on the liposomes, while the destabilization effect of the amphiphilic polymer PHEA-*g*-PDTC is weaker. The destabilization of PHEA results in a sharp increase in the permeability of the liposome membrane, deformation of vesicles, leakage of vesicles, and aggregation of vesicles after leakage. The existence of hydrogen bonding between polymers and liposome membranes is the main reason for inducing destabilization. The semiflexibility of the polymer backbone and the spacer connecting the −OH group and the backbone play a dominant role in the interaction between the polymers and the vesicles.

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