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Full Paper

Water-Soluble Poly(ε-caprolactone)-Paclitaxel Prodrugs Toward an Efficient Drug Delivery System

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In this paper, poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)) was prepared via a thio-bromo click reaction between mercaptosuccinic acid and poly(α-bromo-ε-caprolactone). It is readily soluble in aqueous solutions (pH 5.5–9.8) due to the presence of carboxylic groups in each repeating unit. A series of water-soluble P(α-C_2CL)-paclitaxel prodrugs with high drug contents (up to 41.4 wt-%) was prepared by esterification. Meanwhile, methyl tetrazolium (MTT) assays showed that P(α-C_2CL)-paclitaxel prodrugs exhibited a high antitumour effect on A549 and MCF-7 cells. These prodrugs have appeared as a highly versatile and potent platform for cancer therapy.

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Introduction

The efficacy of many potent and promising anticancer drug molecules is limited by their poor water solubility, and highly cytotoxic side effects,[1,2] thus necessitating an efficient way of systemic transportation. Self-assembled nanovehicles (e.g. micelles,[3,4] liposomes,[5] nanoparticles,[6,7] and polymersomes[8]) encapsulating hydrophobic drugs have been documented as a possible strategy to give improved efficacy of chemotherapeutic drugs delivery to solid tumours and reducing drug release to non-cancerous tissue. However, these nanovehicles tend to dissociate and release encapsulated drug upon intravenous administration.[9,10] In addition, dosage of the drug in nanoparticles or liposomes are not allowed to exceed 10 %[11,12] to minimize the initial drug release in the blood compartments. In contrast, the conjugation of a drug with a polymer forms a so-called 'polymeric prodrug' that reduces the potential for gradual leakage of the drug from the delivery system because of the covalent linkages between the polymers and drug molecules; this has gained tremendous attention for cancer therapy.[13–16] Polymeric prodrugs showed an increased water solubility of hydrophobic drugs, thus, enhancement of drug bioavailability. On one hand they preserved the drugs activity during circulation, and transported it to the target organ or tissue; on the other, they reduced the antigenic activity of the drug and exhibited a less pronounced immunological body response. They enabled passive or active targeting of drugs to tumour tissues and cells.[17–19] So far, numerous polymeric prodrugs have been successfully approved for different phases of clinical trials during the past decades.[13]

Polymeric prodrugs based on biocompatible water-soluble polymers such as poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA)[20,21] and poly(ethylene glycol) (PEG)[22] have received tremendous interest for targeted cancer therapy. Yet, PHPMA and PEG are non-biodegradable and those polymers with a high molecular weight ($M_w > 45$ kDa) are not readily excreted from the body. Polymeric drugs with low molecular weights, however, exhibit relatively short circulation time and poor accumulation in tumours in vivo.[23] Thus, steadily increasing attention has been paid to polymeric prodrugs based on fully biodegradable and water-soluble polymers in the past few years. For example, polypeptide materials (i.e. poly(glutamic acid), poly(L-γ-glutamylglutamine), poly(hydroxyethyl-L-asparagine), poly(hydroxyethyl-L-glutamine)) are able to degrade in vivo into their respective amino acids that can be metabolized by physiological pathways.[24–26] Aliphatic polyesters (i.e. poly(ε-caprolactone), poly(D- or L-lactic acid), and polyglycolic) exhibit promising biocompatibility and are able to degrade through hydrolyzation and/or enzymatic degradation. Poly(ε-caprolactone) (PCL) is a type of aliphatic polyester possessing good biodegradability, biocompatibility, non-toxicity, and high permeability to drugs. PCL is hydrophobic and highly crystalline and it is more resistant to hydrolytic degradation compared with polylactide and polyglycolide.[27–30] However, PCL based prodrugs with water solubility have been rarely studied due to their hydrophobicity and absence of functional moieties, which allow for introducing bioactive moieties. To address this issue, reactive hydrophilic groups (i.e. hydroxy,[31] amino,[32] and carboxylic acid[33]) have been incorporated into PCLs to improve their water solubility and biocompatibility for the preparation of novel fully biodegradable and water-soluble polymeric prodrugs.

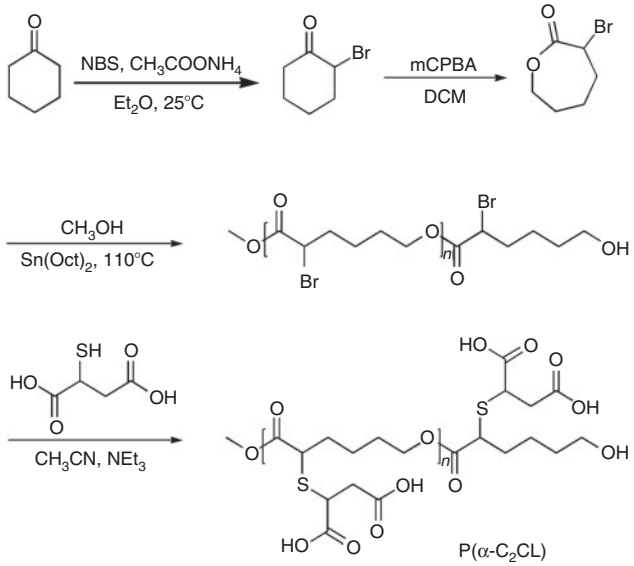
In this contribution, we report a series of poly(ε-caprolactone)-*graft*-carboxylic/paclitaxel (P(α-C_2CL)/PTX) prodrugs which were prepared from water soluble and biodegradable PCL bearing

α-carboxyl groups. Poly(ε-caprolactone)-*graft*-carboxylic (P(α-C_2CL)) was synthesized by ring-opening polymerization of α-bromo-ε-caprolactone and subsequent postpolymerization by a thio-bromo click reaction. It is water soluble, non-toxic, and amendable to conjugate with paclitaxel by esterification, which makes P(α-C_2CL) a unique candidate towards an efficient drug delivery system.

Results and Discussion

Polymer Synthesis

Poly(α-bromo-ε-caprolactone) and P(α-C_2CL) were synthesized by following the procedure as shown in Scheme 1. Poly(α-bromo-ε-caprolactone) was prepared in a multistep route by following a reported procedure.[34] Cyclohexanone was first converted into α-bromocyclohexanone through a substitution reaction with *N*-bromosuccinimide (NBS). Subsequently, the α-bromo-ε-caprolactone monomer was prepared by the Baeyer–Villiger oxidation of α-bromocyclohexanone in the presence of *m*-chloroperoxy benzoic acid. The monomer underwent a ring-opening polymerization using $Sn(Oct)_2$ as the catalyst and methanol as the initiator, yielding a bromo-substituted PCL (Poly(α-bromo-ε-caprolactone)), which was then reacted with thiomalic acid in acetonitrile in the presence of triethylamine as the catalyst via a thio-bromo click reaction to yield P(α-C_2CL). Poly(α-bromo-ε-caprolactone) with different number average molecular weights and molecular weight distributions were synthesised by controlling the initial monomer to initiator ratio (Table 1). In the ^{1}H NMR spectrum (Fig. 1), the integration ratio of proton resonances at 4.16 and 3.8 ppm (Ha) was used to calculate the degree of polymerization (DP) of poly(α-bromo-ε-caprolactone). The successful preparation of P(α-C_2CL) was verified by ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy and gel permeation chromatography (GPC). As shown in Fig. 2, the chemical shift at 3.75 ppm (H_g) was assigned to the characteristic proton of the CH group of thiomalic acid adjacent to the thioether. Integration of these characteristic protons (H_g, H_f) demonstrated the quantitative reaction between thiomalic acid and bromo-substituted PCL, indicating high fidelity of the click reaction. In the FTIR spectra (Fig. 3b), a large broad peak from 2500 to 3500 cm^{-1}, corresponding to the carboxy absorption, emerged after the click reaction, which further confirmed the successful preparation of P(α-C_2CL). In addition, the successful preparations of the polymer was also confirmed by the GPC analyses, as shown in Fig. 4. Unimodal peaks with a low-molecular-weight distribution were clearly observed and the molecular weight of the carboxyl-substituted PCL increased appreciably compared with that of poly(α-bromo-ε-caprolactone), suggesting that the carboxy groups were successfully grafted to the PCL backbone and the polymer did not degrade after the click reaction.



Scheme 1. The synthetic route of poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)).

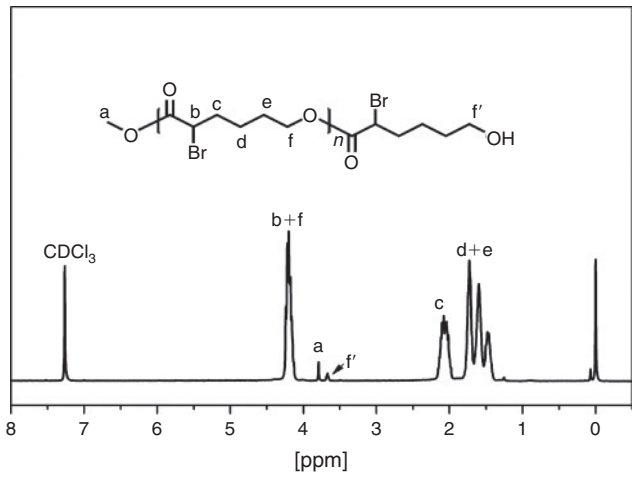


Fig. 1. ^{1}H NMR spectrum of poly(α-bromo-ε-caprolactone) (P(α-BrCL)) in $CDCl_3$.

Table 1. $Sn(Oct)_2$ mediated ring-opening polymerization of α-bromo-ε-caprolactone

Polymerization	M_0/ I_0^A	DP_n^B	$M_{n,theor}^C$ [g mol^{-1}]	$M_{n,GPC}^D$ [g mol^{-1}]	M_w/ M_n^D	Yield [%]
1	30	28	5800	7300	1.24	94
2	50	47	9700	15300	1.15	95
3	80	77	15400	23600	1.27	96
4	120	115	23200	34500	1.31	95

[A]Ratio of monomer to initiator.
[B]Degree of polymerization determined by ^{1}H NMR spectroscopy.
[C]Theoretical number-average molecular weight.
[D]$M_{n,GPC}$ was determined by GPC (with polystyrene standards).

Water Solubility of P(α-C_2CL)

P(α-C_2CL)s (10 mg) with different degrees of polymerization (DP = 28, 47, 77, 115) were dissolved in NaOH aqueous solution (2 mL, pH 9.8) by ultrasonicating for 10 min and standing for 24 h at room temperature (Fig. 5, A_1–A_4). When regulating the pH value to 7.8 by addition of HCl, all polymer solutions became turbid (Fig. 5, B_1–B_4). P(α-C_2CL) gradually precipitated when further decreasing the pH value below 4.4 (Fig. 5, C_1–C_4). Meanwhile, the GPC traces showed unimodal peaks with low-molecular-weight distributions and a similar retention time of P(α-C_2CL) under basic and neutral conditions, indicating that the polymers are relatively stable under basic conditions (Fig. 4).[35] The water solubility of P(α-C_2CL) with different main-chain length and at various pH values are summarised in Table 2. These results indicate that P(α-C_2CL)s are readily dissolved in aqueous solutions when pH > 8.5 because of the deprotonation of carboxylic groups.[36] The effect of the DP on their water solubility is not noticeable.

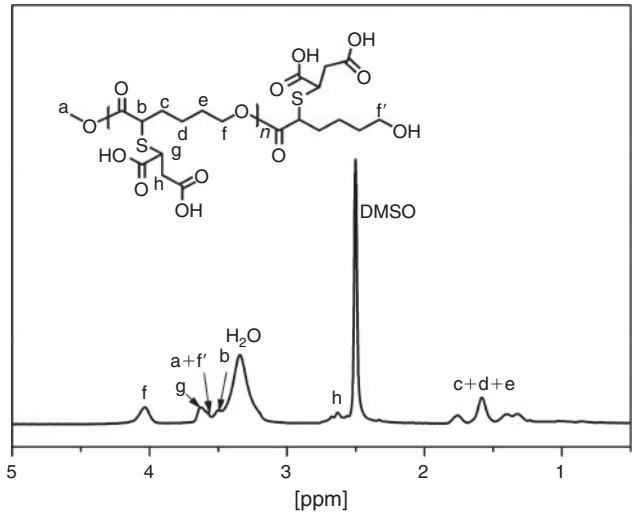


Fig. 2. ^{1}H NMR spectrum of poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)) in DMSO-d_6.

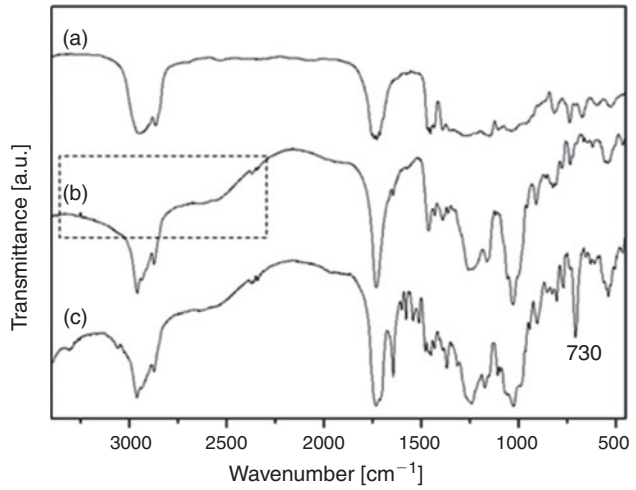


Fig. 3. FTIR spectra of (a) poly(α-bromo-ε-caprolactone) (P(α-BrCL)), (b) poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)), and (c) poly (ε-caprolactone)-*graft*-carboxylic acid–paclitaxel prodrugs (P(α-C_2CL-PTX)).

Synthesis of P(α-C_2CL)-PTX

P(α-C_2CL)-paclitaxel prodrugs (P(α-C_2CL)-PTX) were prepared by esterification between the carboxylic acid side chains of P(α-C_2CL) and paclitaxel (PTX) in the presence of dicyclohexylcarbodiimide (DCC) (Scheme 2). Successful preparation of P(α-C_2CL)-PTX was verified by FTIR, ^{1}H NMR, and UV-vis spectroscopy. As shown in Fig. 3c, P(α-C_2CL)-PTX exhibits several new characteristic absorptions attributed to the benzene rings of PTX, such as the skeleton vibration at 1600, 1585, 1500, and 1450 cm^{-1}, and the bending vibration at 730 cm^{-1}. In the ^{1}H NMR spectrum (Fig. 6), P(α-C_2CL)-PTX shows characteristic resonances of paclitaxel moieties at 7.0–8.2 ppm. Notably, the GPC curves of P(α-C_2CL)-PTX exhibited unimodal peaks, these peaks did not overlap with the signal of the free PTX (Fig. S4, Supplementary Material). The results revealed that PTX was successfully grafted to the P(α-C_2CL) backbone and the polymer-PTX prodrug did not degrade after esterification. In addition, unreacted PTX was removed completely. In the UV-vis spectra (Fig. 7), P(α-C_2CL)-PTX shows the same absorption band at 274 nm as that of PTX in a 1/2 (v/v) methanol/DMF solution.

PTX Loading Efficiency and Water Solubility of P(α-C_2CL)-PTX

The concentration of PTX was linearly correlated with the absorbance at 274 nm in the range of 0.5×10^{-5} to 3.25×10^{-5} g mL^{-1} (the linear regression equation was $A = 0.70563C + 0.09698$, $r = 0.9982$). Therefore, the PTX contents in the P(α-C_2CL)-PTX prodrugs were determined by UV-vis spectroscopy and summarised in Table 3. A single narrow size distribution is observed and the average diameter is 20 nm by dynamic light scattering (DLS) (Fig. S3, Supplementary Material). The average diameter determined was attributed to the pH of the solution of P(α-C_2CL)-PTX being slightly lower than neutral, which resulted in spontaneous self-assembly of P(α-C_2CL)-PTX. In order to demonstrate whether the P(α-C_2CL)-PTX aggregated in aqueous solution at a lower pH value, we measured the solution properties of P(α-C_2CL)-PTX at different pH values. When regulating the pH value from 8.0–7.0 to 7.0–6.0, the transmittance of P(α-C_2CL)-PTX solutions

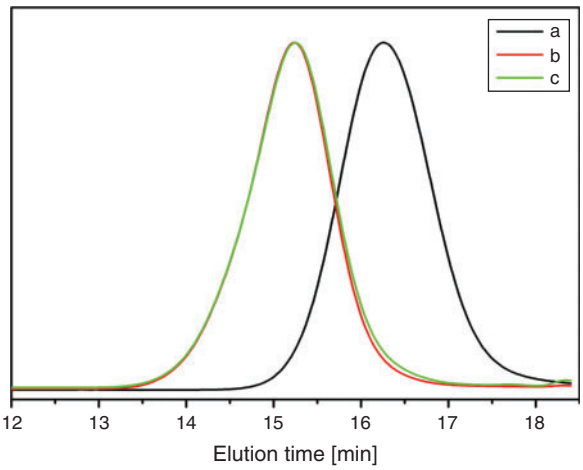


Fig. 4. Gel permeation chromatography traces of (a) poly(α-bromo-ε-caprolactone) (P(α-BrCL)), (b) poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)), and (c) P(α-C_2CL) under basic condition standing for 24 h.

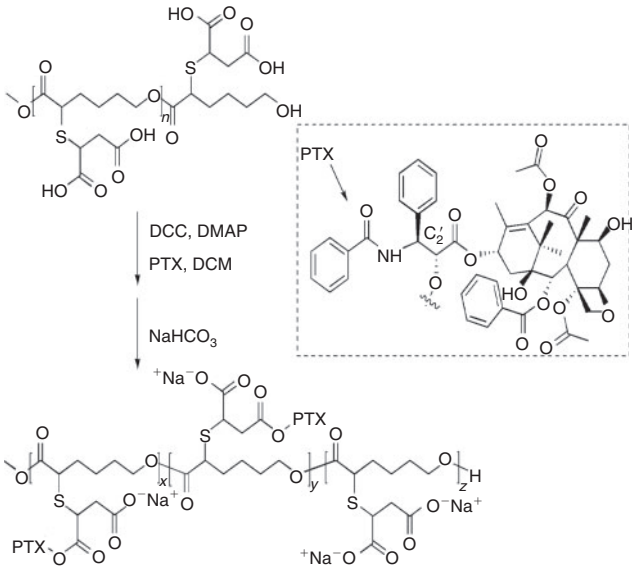
Fig. 5. Digital photos for the aqueous solution of P(α-C_2CL)$_{28}$ (1), P(α-C_2CL)$_{47}$ (2), P(α-C_2CL)$_{77}$ (3) and P(α-C_2CL)$_{115}$ (4). P(α-C_2CL): poly(ε-caprolactone)-*graft*-carboxylic acid. (A: at pH 8.5–9.8; B: at pH 5.5–7.8; C: at < pH 4.4).

Table 2. Water solubility of poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)) with various degree of polymerization at different pH values

Polymers	Solubility		
	pH 2.5, 3.2, 4.4	pH 5.5, 6.7, 7.8	pH 8.5, 9.3, 9.8
P(α-C_2CL)$_{28}$	insoluble[A]	slightly soluble[B]	soluble[C]
P(α-C_2CL)$_{47}$	insoluble	slightly soluble	soluble
P(α-C_2CL)$_{77}$	insoluble	slightly soluble	soluble
P(α-C_2CL)$_{115}$	insoluble	slightly soluble	soluble

[A]Insoluble (<1 mg mL^{-1}).
[B]Slightly soluble (1–5 mg mL^{-1}).
[C]Soluble (>5 mg mL^{-1}).

decreased sharply at low pH values (Fig. S5, Supplementary Material). Furthermore, the water solubility of P(α-C_2CL)-PTX decreased as the PTX contents increased (Table 3). In order to ensure relatively high drug loading and good water-solubility of the prodrugs, the drug-loading efficacy was relatively suitable in the range of 10 to 40 %.



Scheme 2. The synthetic route of P(α-C_2CL)-PTX conjugate.

In Vitro Cytotoxicity

The in vitro cytotoxicity of P(α-C_2CL) was evaluated by methyl tetrazolium (MTT) assay. Two cell lines (i.e. MCF-7 and A549 cells) were applied. As shown in Fig. 8, the viabilities of MCF-7 and A549 cells treated with P(α-C_2CL) were in the range of 80 to 110 % at all test concentrations up to 10 mg mL^{-1} after 72 h incubation, revealing the low toxicity and good compatibility of the polymer to the cells.

The in vitro antitumour activity of the P(α-C_2CL)-PTX conjugate was also studied in MCF-7 and A549 cells. The cell viabilities were evaluated after 24, 48, or 72 h incubation with the P(α-C_2CL)-PTX conjugate, and free PTX was used as a control. As shown in Fig. 9, the P(α-C_2CL)-PTX conjugate exhibited dose and time dependent cell proliferation inhibition for both MCF-7 and A549 cells. The viabilities of MCF-7 and A549 cells treated with the P(α-C_2CL)-PTX conjugate were

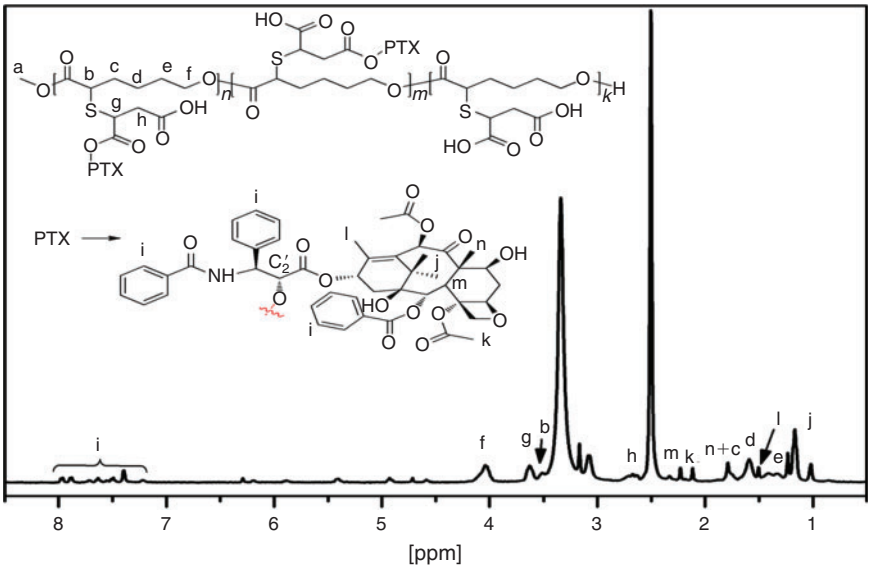


Fig. 6. ^{1}H NMR spectrum of poly(ε-caprolactone)-*graft*-carboxylic acid–paclitaxel prodrugs (P(α-C_2CL-PTX)) in DMSO-d_6.

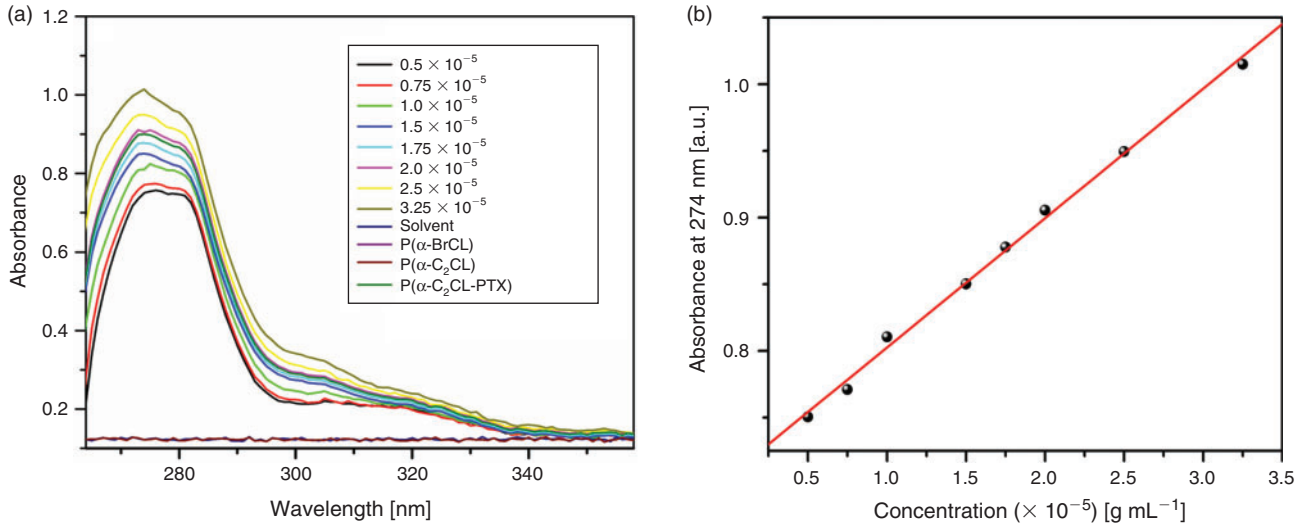


Fig. 7. (a) UV-vis spectra of PTX in DMF/methanol at different concentrations and poly(α-bromo-ε-caprolactone) (P(α-BrCL)), poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)), and poly(ε-caprolactone)-*graft*-carboxylic acid–paclitaxel prodrugs (P(α-C_2CL-PTX)) in DMF/methanol. (b) Absorption intensity of P(α-C_2CL)-PTX at 274 nm in their DMF/methanol solutions of different concentrations.

Table 3. Water solubility of poly(ε-caprolactone)-*graft*-carboxylic acid–paclitaxel (P(α-C_2CL-PTX)$_{77}$) prodrugs with different paclitaxel (PTX) contents

Prodrugs	PTX content [wt-%]		Water solubility [mg mL^{-1}]
	Calculated	Observed	
P(α-C_2CL-PTX)$_{77-1}$	13.8	12.1	>250
P(α-C_2CL-PTX)$_{77-2}$	24.2	20.3	>200
P(α-C_2CL-PTX)$_{77-3}$	39.4	31.5	>150
P(α-C_2CL-PTX)$_{77-4}$	44.8	35.4	>100
P(α-C_2CL-PTX)$_{77-5}$	53.2	41.4	<50

in the range of 74 to 100 % at all test concentrations up to 10 μg PTX equiv. mL^{-1} after 24 h incubation, indicating that the P(α-C_2CL)-PTX conjugate displayed almost no antitumour activity within 24 h. After 48 h incubation, the P(α-C_2CL)-PTX conjugate displayed a slightly higher drug efficacy than that of free PTX in MCF-7 cells at a concentration of 4 μg PTX equiv. mL^{-1} and lower. When drug concentrations were higher than 4 μg PTX equiv. mL^{-1}, the antitumour activity of the PTX prodrug became lower than that of free PTX. The maximal half inhibitory concentrations (IC_{50}) were 3.45 and 3.89 μg PTX equiv. mL^{-1} for the P(α-C_2CL)-PTX conjugate and free PTX, respectively. After 72 h incubation, the P(α-C_2CL)-PTX conjugate displayed a slightly higher drug efficacy then free PTX in MCF-7 cells between a concentration of 0.31 and 5.76 μg PTX equiv. mL^{-1}. The IC_{50} of the P(α-C_2CL)-PTX conjugate and free PTX were 0.694 and 0.95 μg PTX equiv. mL^{-1}, respectively. In the A549 cell line, the P(α-C_2CL)-PTX conjugate exhibited a lower antitumour effect than free PTX. After 48 h incubation, the IC_{50} of the P(α-C_2CL)-PTX conjugate and free PTX were 3.15 and 1.81 μg PTX equiv. mL^{-1}, respectively. However, the IC_{50} of the P(α-C_2CL)-PTX conjugate was 1.01 μg PTX equiv.

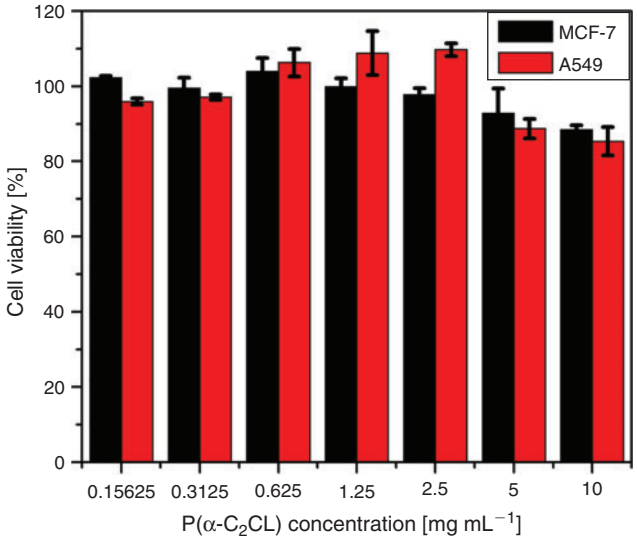


Fig. 8. In vitro cell cytotoxicity of poly(ε-caprolactone)-*graft*-carboxylic acid (P(α-C_2CL)) against A549 and MCF-7 cells at different concentrations after 72 h.

mL^{-1} after 72 h incubation, which was lower than that of free PTX (1.33 μg mL^{-1}). The results show that the MCF-7 cells were much more sensitive to the PTX prodrug and free PTX than A549 cells after 72 h incubation. Meanwhile, the prodrug exhibited a lower antitumour efficacy as compared with the free PTX.[37,38] These results suggest that the PTX-polymers release PTX in a pristine state and it retains its potent therapeutic activity, as such P(α-C_2CL)-PTX prodrugs possess great anti-tumour ability towards an efficient drug delivery system.

Conclusions

A new family of poly(ε-caprolactone)s bearing carboxylic pedants (P(α-C_2CL)) was synthesized via a combination of ring-opening polymerization and thio-bromo click reaction. The anticancer drug (i.e. paclitaxel) was successfully conjugated to P(α-C_2CL) via esterification with a high PTX content (up to 40 wt-%). P(α-C_2CL)-PTX are readily dissolved in aqueous solutions (> 50 mg mL^{-1}) when the carboxylic side-chains are deprotonated. P(α-C_2CL)-PTX prodrugs showed potent cellular growth inhibition abilities in MCF-7 (IC_{50} = 0.694 μg PTX equiv. mL^{-1}) and A549 (IC_{50} = 1.01 μg PTX equiv. mL^{-1}) cell

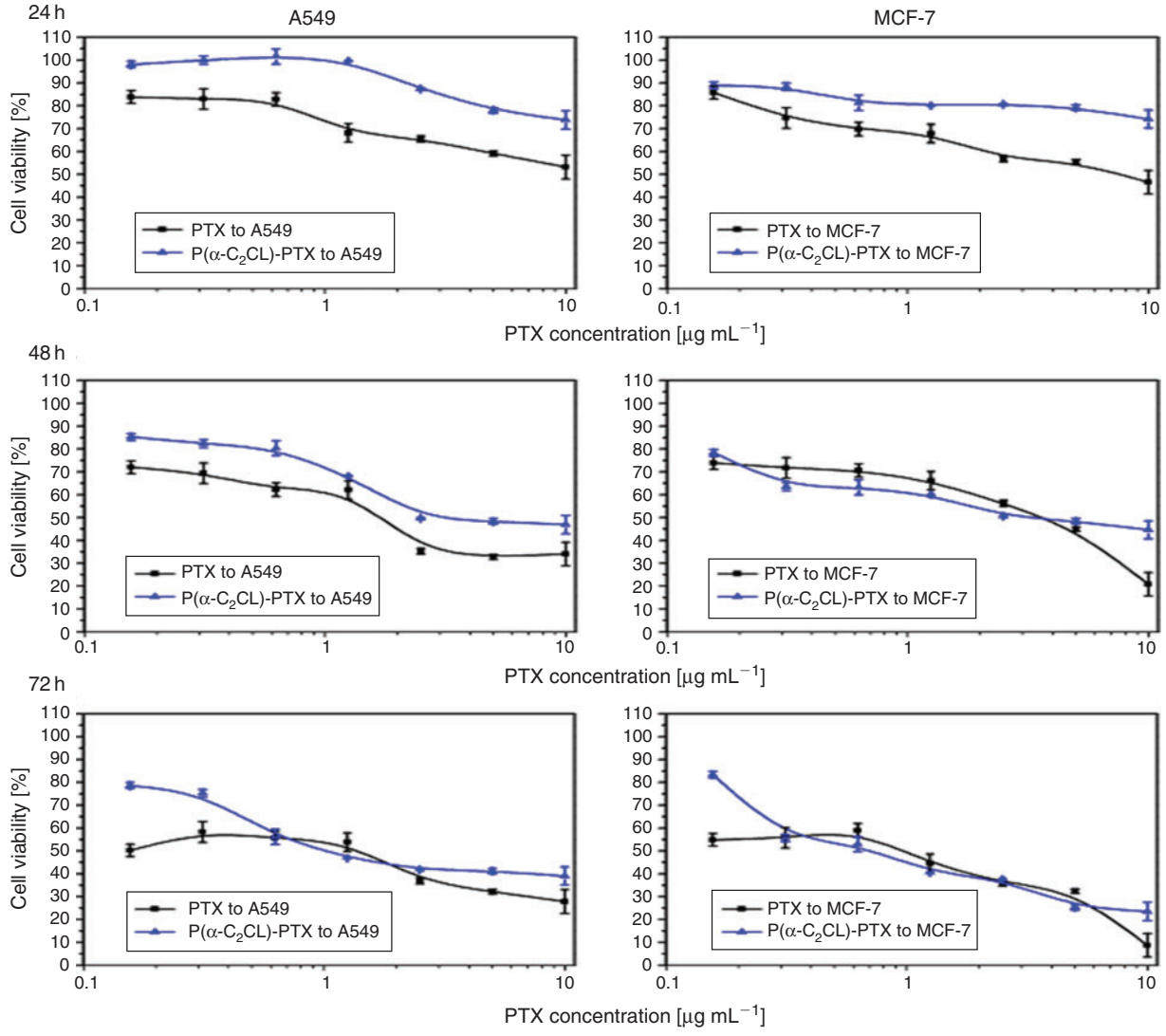


Fig. 9. In vitro cell viability of A549 and MCF-7 cells against free paclitaxel (PTX) and the poly(ε-caprolactone)-*graft*-carboxylic acid–paclitaxel prodrug (P(α-C_2CL-PTX)) at different concentrations and incubation time.

lines after 72 h incubation. Our work indicates that the P(α-C_2CL)-PTX will be a great potential drug delivery system for cancer therapy.

Experimental

Instruments and Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 400 M. $CDCl_3$ and DMSO-d_6 were used as the solvents. Average molecular weights (M_n) and polydispersities (PDI) were measured on a PL-GPC120 setup equipped with a column set consisting of two PL gel 5 μm MIXED-D columns (7.5 × 300 mm, effective molecular weight range of 0.2–400.0 kg mol^{-1}) using *N*,*N*-dimethylformamide that contained 0.01 M LiBr as the eluent at 80°C at a flow rate of 1.0 mL min^{-1}. Narrowly distributed polystyrene standards in the molecular weight range of 0.5–7500.0 kg mol^{-1} (PSS, Mainz, Germany) were utilised for calibration. FTIR spectra were recorded on a Perkin–Elmer Spectrum one FTIR spectrophotometer, five scans were signal-averaged with a resolution of 10 cm^{-1} at room temperature. Samples were prepared by dispersing the complexes in KBr and compressing the mixtures to form disks. UV-vis spectra were measured using a PE Lambda 20 spectrophotometer. UV irradiation was carried out with a 300 W high-pressure mercury lamp coupled with UV filters (<360 nm). The size of particles was measured by DLS with a vertically polarized He–Ne laser (DAWNEOS, Wyatt Technology, USA) at a fixed scattering angle of 90° and at a constant temperature of 25°C. The sample was filtered through Millipore membranes with pore sizes of 0.45 μm before measurement.

Methods

Synthesis of Poly(α-bromo-ε-caprolactone) by Ring-Opening Polymerization

α-Bromo-ε-caprolactone (1.00 g, 0.005 mol), toluene (5 mL), methanol (1.7 mg, 0.52 mmol), and $Sn(Oct)_2$ (21 mg, 0.5 mmol) were added into a H_2O-free polymerization flask under nitrogen, followed by stirring at 110°C for 48 h. The solvents were then removed by rotary evaporation and the residue was dissolved in CH_2Cl_2. The polymer/CH_2Cl_2 solution was precipitated from cold methanol to yield a colourless, transparent, and sticky solid product and dried under vacuum overnight at room temperature (obtained: 0.95 g, yield: 95 %). δ_H (400 MHz, $CDCl_3$) 1.48–2.14 (m, 6H, –$CH_2CH_2CH_2$–), 3.7 (m, 2H, –C*H*$_2$OH), 3.8 (s, 3H, –CH_3), 4.16 (m, 3H, –CHBr–, –$COOCH_2$–). δ_C (400 MHz, $CDCl_3$) 169.65, 65.44, 45.64, 45.62, 34.26, 27.70, 23.72.

Synthesis of P(α-C_2CL) by a Thio-Bromo 'Click' Reaction

Triethylamine (6 mL) was added dropwise to an acetonitrile solution (10 mL) containing thiomalic acid (1.00 g, 0.006 mol) under N_2, followed by slow addition of acetonitrile solution containing poly(α-bromo-ε-caprolactone) (0.50 g, 0.041 mol). The mixture was stirred at room temperature for 24 h. The solvent was then removed and the residue was dissolved in deionized water by regulating the pH value to 8.5–9.0 with 2 M NaOH aqueous solution. The polymer aqueous solution was extracted with CH_2Cl_2 and ethyl acetate three times to remove the impurities. The polymer was precipitated by regulating the pH to 3.5 with 6 M HCl(aq), filtered off, and freeze-dried to yield a white sticky solid (obtained: 0.51 g, yield: 77 %). δ_H (400 MHz, d_6-DMSO) 1.23–1.75 (m, 6H, –$CH_2CH_2CH_2$–), 2.67 (m, 2H, –C*H*$_2$COOH–), 3.6 (s, 3H, –CH_3), 3.7 (m, 2H, –C*H*$_2$OH), 3.75 (m, 1H, –C*H*COOH–). δ_C (400 MHz, d_6-DMSO) 173.4, 173.1, 172.5, 64.8, 46.6, 43.1, 37.5, 31.6, 31.1, 23.4

Synthesis of P(α-C_2CL)-PTX

Under a N_2 atmosphere, a mixture of P(α-C_2CL) (0.1 g, 0.45 mmol), DCC (0.024 g, 0.085 mmol), and catalytic amounts of dimethylaminopyridine (DMAP) in DMF (2 mL) was stirred at 0°C for 0.5 h. PTX (0.065 g, 0.08 mmol) was then added and the mixture kept stirring at room temperature for 24 h. The consumption of PTX was monitored by thin-layer chromatography. The solvent was removed at reduced pressure and the residue was dissolved in deionized water (50 mL) by regulating the pH value to 8.5–9.0 with 2 M NaOH aqueous solution. The polymer aqueous solution was extracted with CH_2Cl_2 and ethyl acetate three times to remove the impurities. The polymer was precipitated by regulating the pH to 3.5 with 6 M HCl(aq), filtered off, and freeze-dried to yield a white sticky solid (obtained: 0.12 g, yield: 73 %). δ_H (400 MHz, d_6-DMSO) 1.23–1.75 (m, 6H, –$CH_2CH_2CH_2$–), 2.67 (m, 2H, –C*H*$_2$COOH–), 3.6 (s, 3H, –CH_3), 3.7 (m, 2H, –C*H*$_2$OH), 3.75 (m, 1H, –C*H*(COOH)–), 4.0 (m, 3H, –$COOCH_2$– and –COCH–), 7.3–8.4 (m, 15H, Ar-H).

Water Solubility of P(α-C_2CL)

The effect of degree of polymerization (DP = 28, 47, 77, 115) and pH (pH = 2.5, 3.2, 4.4, 5.5, 6.7, 7.8, 8.5, 9.3 and 9.8) on the water-solubility of P(α-C_2CL) was determined by sonicating P(α-C_2CL) (10 mg) in a 10 mL aqueous solution for 10 min at room temperature. The pH value was adjusted using NaOH or HCl aqueous solutions.

PTX Loading Efficiency and Water Solubility of P(α-C_2CL)-PTX

Loading Efficiency

The PTX-loading efficiency of the P(α-C_2CL)-PTX prodrug was determined by UV-vis spectroscopy. Briefly, PTX was dissolved in a methanol/DMF solution (1/2, v/v) to yield a series of standard solutions at certain concentrations (i.e. 0.5×10^{-5}, 0.75×10^{-5}, 1.0×10^{-5}, 1.5×10^{-5}, 1.75×10^{-5}, 2.0×10^{-5}, 2.5×10^{-5}, 3.25×10^{-5} g mL^{-1}). A calibration curve that correlates the absorbance at 274 nm with the concentration of PTX was constructed. The concentration of PTX in the P(α-C_2CL)-PTX) aqueous solution was obtained using the calibration curve. Thus, the estimated PTX-loading efficiency was calculated using the following equation:

$$\text{Percentage drug loading} = (\text{concentration of PTX in P}(\alpha\text{-}C_2\text{CL})\text{-PTX}/\text{concentration of P}(\alpha\text{-}C_2\text{CL})\text{-PTX}) \times 100\,\% \quad (1)$$

Water Solubility

P(α-C_2CL)-PTX was first dissolved in a $NaHCO_3$ aqueous solution, followed by dialyzing against distilled water and lyophilization to obtain a solid product for the water-solubility measurement. Typically, 100 mg of P(α-C_2CL)-PTX and deionized water were mixed in a 10 mL flask by ultrasonicating for 10 min. The volume of the solution was measured to calculate the water solubility. The size of P(α-C_2CL)-PTX in aqueous solution was measured by DLS. The influence of pH on the water solubility of P(α-C_2CL)-PTX was determined by sonicating P(α-C_2CL)-PTX (100 mg) in a 10 mL aqueous solution for

10 min at room temperature. The pH value was adjusted using HCl aqueous solutions.

Cell Culture

A549 (human pulmonary carcinoma) and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose levels containing 10 % fetal bovine serum, supplemented with 50 U mL^{-1} penicillin and 50 U mL^{-1} streptomycin, and incubated at 37°C in 5 % CO_2 atmosphere.

In Vitro Cytotoxicity Assay

The in vitro cytotoxicity was assessed with a MTT viability assay against A549 and MCF-7 cells. A549 cells and MCF-7 cells were seeded in 96-well plates at 10000 cells per well in 100 μL of DMEM medium and incubated for 24 h, followed by removing the culture medium and adding P(α-C_2CL), PTX, P(α-C_2CL)-PTX (200 μL in complete DMEM medium) at different concentrations. The cells were subjected to MTT assay after being incubated for another 24, 48, or 72 h. At the end of the experiments, 20 μL of MTT solution (5 mg mL^{-1} in phosphate buffered saline) was added to each well. The plate was returned to the incubator. After 4 h, the MTT solution was carefully removed from each well, and the MTT-formazan generated by live cells was dissolved in 200 μL of DMSO. The absorbance of the solution was measured on a Bio-Rad 680 microplate reader at 492 nm. Cell viability (%) was calculated by $(A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ denote the absorbance of the sample well and control well, respectively. Data are presented as average ± s.d. ($n = 3$).

Supplementary Material

The ^{13}C NMR spectrum and size distribution profiles of polymers (Figs S1–S3), GPC curves of PTX-polymers and free PTX (Fig. S4), and digital photos of the aqueous solution of PTX-polymers (Fig. S5) are available on the Journal's website.

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