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Synthesis and solution properties of pH responsive methoxy poly(ethylene glycol)-*b*-poly(γ-amino-ε-caprolactone)

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Experimental

1. Materials and methods

1.1. Materials

Monomethoxy-poly(ethylene glycol) (mPEG, Mn=2000, 99%) were purchased from Aldrich and dried by an azeotropic distillation in toluene prior to use. Triphosgene, hydrobromic acid in glacial acetic acid (33 wt%) and trifluoroacetic acid (TFA, 99%) and tin (II)-2-ethylhexanoate [$Sn(Oct)_2$, 95%] were bought from Sigma and used

without further purification. Cell culture media RPMI1640 and PBS were purchased from Invitrog. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was provided by Sigma. Human dermal fibroblast line (HF， supplied by school of biotechnology, East China University of Science and Technology). Tetrahydrofuran (THF) and toluene were dried and distilled in the presence of sodium. Other reagents were commercially available and used as received.

1.2. Synthesis of mPEG-*b*-PCABCL

γ-(carbamic acid benzyl ester)-ε-caprolactone (γCABεCL) monomer was synthesized according to the previous study in our lab.[1] The mPEG-*b*-PCABCL copolymers were synthesized by ring opening polymerization of γCABεCL in the presence of mPEG as macroinitiator and $Sn(Oct)_2$ as catalyst. In a typical procedure, a schlenk flask containing a stir bar was fitted with a septum, flame-dried under vacuum and filled with argon. γCABεCL (2.40 g, 9.13 mmol) and mPEG (0.30 g, 0.15 mmol) were added under the protection of argon, and then schlenk flask was connected to a Schlenk line. Meanwhile, an exhausting-refilling process was repeated for several times. A total of 72.4 μL of $Sn(Oct)_2$ in dry toluene stock solution was added under argon atmosphere by using a microliter syringe, and the exhausting-refilling process was conducted again to remove the toluene. The schlenk flask was sealed under vacumm and immersed in an oil bath at 130 °C for 24 h. The cooled product was dissolved in 6 mL dichloromethane (DCM), recovered by precipitation into an excessive amount of cold diethyl ether. The precipitates were filtered out and dried

under vacuum at room temperature.

1.3. Removal of the protective groups

The amino groups of mPEG-*b*-PACL copolymers were regenerated by hydrolysis of the Cbz groups in TFA using a 33 wt% hydrobromic acid solution in glacial acetic acid. Typically, the protected copolymer mPEG-*b*-PCABCL (1.00 g) was first dissolved in 10 mL dichloromethane under stirring in a two-necked round-bottomed flask in an ice-water bath, 2 mL TFA were added and followed by dropwise of a 6 mL 33 wt% solution of HBr in glacial acetic acid, then the mixture was vigorously stirred at 0 °C for 1.5 h. The resultant solution was concentrated to remove the solvent and the residue was dissolved in 10 mL DMF and then poured into an excess of cold diethyl ether. Then The precipitates were dissolved in 5 mL DMF again and treated with 2 mL triethylamine (TEA) for 2 h at room temperature, followed by dialysis against water for 24 h before freeze drying to obtain mPEG-*b*-PACL with free pendant amine groups. (Yield: 65 %)

2. Characterization

2.1. Polymer characterization

The molecular weight and polydispersity of mPEG-*b*-PCABCL were measured by size exclusion chromatography (SEC, Waters1515 HPLC). THF was used as the eluent at a flow rate of 1.0 $mL \cdot min^{-1}$. The column system was calibrated by monodispersed standard polystyrene. However, the molecular weight and

polydispersity of mPEG-*b*-PACL was determined by size exclusion chromatography (SEC, Perkin-Elmer Series 200 system). Dimethylformamide (DMF) containing 0.01 $mol \cdot L^{-1}$ lithium bromide was used as eluent at a flow rate of 1.0 $mL \cdot min^{-1}$. The column system was calibrated by monodispersed standard polyethylene glycol. ^{1}H NMR spectra was performed on a Bruker AV apparatus at frequencies of 400 MHz, using tetramethylsilane (TMS) as internal reference in $CDCl_3$ for mPEG-*b*-PCABCL and deuterated water (D_2O) for mPEG-*b*-PACL at 25 °C.

Fourier transform infrared spectroscopy (FTIR) were recorded on a Nicolet 5700 spectrometer in the range of 4000 to 500 cm^{-1}. The copolymer samples were mixed with KBr powders and pressed into flakes for FTIR measurements.

DSC measurements were carried out on a Perkin Elmer Instrument (TA DSC Q100) from -90 °C to 400 °C under nitrogen atmosphere at a heating rate of 10 $°C \cdot min^{-1}$.

2.2. Study of the solution properties

The pKa of the ACL homopolymers and mPEG-*b*-PACL diblock copolymer were determined by titration on a Radio-meter(AB U93). The samples were first dissolved in H_2O, and the pH value of the solution was adjusted to pH = 2 by HCl (0.01 M), then 0.25 M NaOH solution was added dropwise into the solution until the pH kept almost constant. All the titration measurements were carried out in a titration vessel at 25 °C.

The zeta potential (§) of mPEG-*b*-PACL diblock copolymers were measured on a Zetasizer Nano-ZS (Malvern) at different pH. The solution pH was adjusted by using

NaOH or HCl according to the requirement.

The size and size distribution of the copolymer solutions were determined by DLS measurement at 25 °C using a light scattering spectrophotometer (Brookhaven BI-200SM) with a vertically polarized incident beam at 532 nm supplied by an argon ion laser. All measurements were performed at a fixed scattering angle of 90^{o} and the temperature was controlled at 25 °C. Before measurements, all samples were filtered through a 0.45 μm filter (Millipore) and the copolymer solutions were studied at different pH from 2 to 12 at the concentration of 1 $mg \cdot mL^{-1}$.

Transmission electron microscopy (TEM) images were performed on a transmission electronic microscope instrument (JEOL/JEM-2000E XII) operated at an acceleration voltage of 60 keV. A drop of the copolymer solution was deposited on the copper grid followed by drying at room temperature before observation.

2.3. The cytotoxicity of the copolymers

The cytotoxicity of the copolymers was evaluated by MTT assay. The human dermal fibroblast cells were seeded onto the 96-well plates at a density of 5×10^{3} viable cells/well and cultured to permit cells attachment. After 24 h, the cells were incubated in the culture medium containing different concentrations of copolymer solution. The cells were incubated in the culture medium and maintained in 5% CO_2 at 37 ^{o}C for a further 24 h. Then the culture medium was removed, fresh medium (200 μL) and MTT (20 μL, 5 $mg \cdot mL^{-1}$ in sterile-filtered PBS) were added to each well, and the plates were re-incubated for a further 4 h. Then the supernatant was carefully

removed by centrifugation for 10 min, and 200 μL of DMSO was added in each well. The absorbance of the solution was measured using microplate reader (Bio-Rad 550) at 492 nm to determine the optical density (OD_{492}) value. The cell viability was calculated as follows:

Cell viability (%)= $(OD_{treated}/OD_{control}) \times 100\%$

where $OD_{treated}$ was obtained for the cells treated by the copolymer solution, $OD_{control}$ was obtained for the cells untreated by the copolymer solution, and the other culture conditions were the same. The data were given as mean standard deviation (SD) based on three independent measurements.

References and Notes

1 J. L. Yan, Y. Zhang, Y. Xiao, Y. Zhang and M. D. Lang, *React. Funct. Polym.*, 2010, **70**, 400-407.

Fig. S1 ^{1}H NMR spectra of mPEG-*b*-PCABCL in $CDCl_3$ (a) and mPEG-*b*-PACL in D_2O (b).

Fig. S2 FTIR spectra of mPEG-*b*-PCABCL (a) and mPEG-*b*-PACL (b).

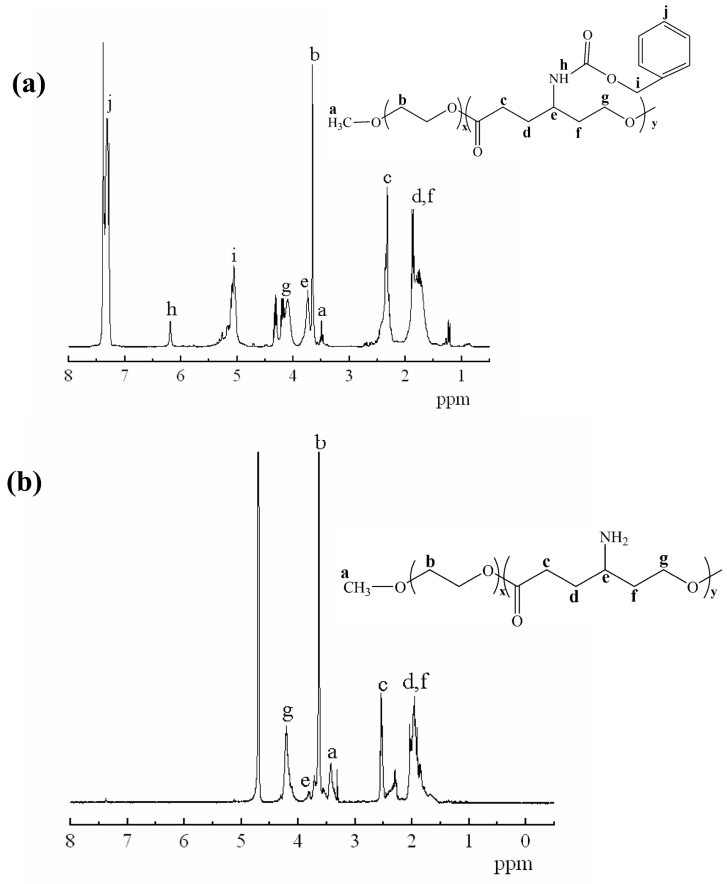

Fig. S1 [1] H NMR spectra of mPEG-*b*-PCABCL in $CDCl_3$ (a) and mPEG-*b*-PACL in D_2O (b).

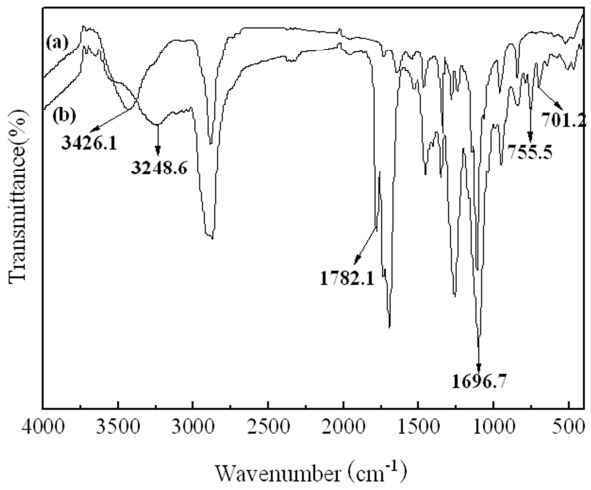

Fig. S2 FTIR spectra of mPEG-*b*-PCABCL (a) and mPEG-*b*-PACL (b).