Synthesis and Self-Assembly of Biodegradable Poly(ethylene oxide)-*b*-Polycaprolactone (PEO-*b*-PCL) Diblock Copolymers

G. Li,[*,**] P. Ghoroghchian[***], W. Qi[**], N. Christian[***], P. R. Frail[**], D. A. Hammer[***], M. J. Therien[**]

[*] Carestream Health Inc.,
1999 Lake Avenue, Rochester, NY 14650-02102, liguizhi@yahoo.com
[**]Department of Chemistry, University of Pennsylvania,
Philadelphia, PA 19104, therien@sas.upenn.edu
[***]School of Engineering and Applied Science, and Institute for Medicine and Engineering,
University of Pennsylvania, Philadelphia, PA 19104

ABSTRACT

A series of amphiphilic poly(ethylene oxide)-*b*-polycaprolactone (PEO-*b*-PCL) diblock copolymers had been synthesized by ring-opening or sequential anionic living polymerization. Nuclear Magnetic Resonance (NMR) and gel permeation chromatography (GPC) were used to characterize these copolymers. All copolymers have polydispersity index (PDI) from 1.14 to 1.37 by GPC. Two self-assembly techniques, film rehydration and organic solvent extraction, had been employed to process polymersomes. The perfect nanostructured polymersomes were processed by freeze-thaw-sonication film rehydration technique. The polymersomes with sizes from a hundred nanometer to several microns were obtained from these copolymers. Polymersomes could only be made from the copolymers with PEO blocks (Mn: 2k-3.8k) and f_{PEO} (11.8-18.8%). Film rehydration is a highly favorable self-assembly method for preparing biodegradable nanostructured polymersomes.

Keywords: nanostructured polymersomes, synthesis, self-assembly, poly(ethylene oxide)-*b*-polycaprolactone (PEO-*b*-PCL) diblock copolymers, biodegradable

1 INTRODUCTION

Polymersomes, vesicles, made from amphiphilic diblock or triblock copolymers, have attracted much attention in the past decade due to their excellent properties over liposomes and micelles [1-3]. Polymersomes exhibited much higher stability than liposomes, phospholipids vesicles [2]. Micelles lack the shell-like character and encapsulated bulk solution phase of a polymersome [1]. Polymersomes not only can encapsulate hydrophobic substances in their membrane shell phase like micelles, but also can entrap water-soluble hydrophilic compounds (drug, vitamin, or fluorophores, etc.) inside of their membrane. Furthermore, size, membrane thickness, and stability of polymersomes could be controlled by selecting block copolymer chemical structure, molecular weight of each block, ratios of hydrophilic block to hydrophobic block, and self-assembly methods [1]. Thus, polymersomes have potential applications in medical devices, drug delivery, cosmetics products, etc. [1,3,4].

2 EXPERIMENTAL

2.1 Materials

ε-caprolactone (ε-CL) was purchased from Aldrich. It was dried over calcium hydride (CaH_2) at room temperature for 48 h and distilled under reduced pressure. Monomethoxyl poly(ethylene oxide) (MePEO) molecular weights of 5000, 2000. 1100 and 750, were supplied by Fluka. MePEO samples (M_n=1100, 2000 and 5000) were purified by dissolving in tetrahydrofuran (THF), followed by precipitating into ether, and dried in vacuum oven (about 10mm Hg) at 40 ºC for one day. MePEO (Mw=750) was used as received. Stannous octonate ($SnOct_2$) (from Sigma) was used as received. Ethylene oxide (EO) was purchased from Aldrich. EO was purified by passage through a tower of potassium hydroxide, followed by condensed onto CaH_2. Naphthalene was recrystallized from ether before use. Other chemicals were commercially available and used as received.

2.2 Polymerization Reactions

Ring-opening polymerization: A certain amount of monomethoxyl poly(ethylene oxide) (MePEO) was filled into a flamed flask under an argon atmosphere. A known volume of caprolactone monomer (with a certain weight ratio to MePEO) was injected into the flask by a syringe. Two small drops of $SnOct_2$ were added to this reactant mixture. The flask was connected to a vacuum line, evacuated, sealed off and placed in oil bath at 130 ºC. A progressive increase on viscosity of the bulk homogeneous mixture was always observed during the polymerization. The copolymers were obtained after 24 h. After cooling at room temperature, the flask was opened. The resulting block copolymers were dissolved in methylene chloride and precipitated into excess of cold methanol/hexane mixture. The white powder products were obtained and dried at 40 ºC under vacuum for more than two days.

Anionic living polymerization: In a flame-dried and argon-purged flask, 30ml of THF, 0.55 ml (10 mmol) of acetonitrile and 5 ml potassium naphthalene/THF solution (1 mmol/ml) were added under argon stream. After being vigorously stirred for 70 min at room temperature (about 20 ºC), this mixture was cooled in an ice-water bath, and a certain amount of distilled EO was added. After 48 h polymerization at room temperature, a part of the reaction product CN-PEO (ca. 5 ml) was taken out for measurement. It was treated by an acetone solution containing some acetic acid, precipitated with an excess amount of diethyl ether and dried in vacuum oven at room temperature. In the second step, a calculated amount of ε-caprolactone in THF (a certain mole ratio of CL/EO) was added to the remaining part of the CN-PEO. After 5-10 min at 0 ºC, the polymerization was quenched by adding excess acetone solution containing a little acetic acid, and the copolymer was recovered by precipitation in diethyl ether and drying in vacuum oven at 40 ºC for about two days.

2.3 Characterizations of Copolymers

^{1}H-NMR (proton-nuclear magnetic resonance) spectra of PEO polymers and copolymers were characterized by using Bruker 300MHz or 500Mhz NMR instruments. Deuterated chloroform ($CDCl_3$) was used as solvent and tetramethylsilane (TMS) as internal standard. Weight-average molecular weight (Mw) values and their polydispersity index (M_w/M_n) were determined by GPC (RAININ HPXL) equipped with two columns (PLgel 5μ Mixed, 300X7.5mm) at room temperature (25 ºC). Dynamic laser scattering and refractive index detector was used for data collection. THF was used as eluent solvent. Poly(ethylene oxide) standards were used to calibrate the molecular weight of the copolymers from their refractive index data.

2.4 Preparation of Polymersomes by Self-assembly

Two self-assembly methods, film rehydration and extraction, were employed to assemble the copolymers into polymersomes. Film rehydration had been used for preparing unbiodegradable polymersomes from PBD-*b*-PEO diblock copolymers.[1,3] The procedures for biodegradable polymersomes preparation from PEO-*b*-PCL copolymers slightly revised and shown as follows. 200μml of 5-10 mg/ml PEO-*b*-PCL copolymer solution in chloroform was uniformly coated on the surface of Teflon plate, followed by evaporation of the solvent under vacuum for overnight. Addition of sucrose solution (250–300 milliosmolar) or PBS buffer and heating at 60°C for 48 h led to spontaneous budding of giant (1–10 μm) biodegradable polymersomes off of the Teflon plate into the aqueous solution. Nile red was incorporated into the polymersomes membrane for confocal laser fluorescence microscopy measurement. Small (<300-nm diameter) unilamellar polymersomes that possess appropriately narrow size distributions were prepared via procedures analogous to those used to formulate small lipid vesicles (sonication, freeze-thaw extraction, and extrusion),[3] The sonication procedure involved placing a sample vial containing the aqueous-based solution and a dried thin-film formulation (of copolymer uniformly deposited on Teflon) into a bath sonicator (Fischer Scientific; Model FS20) with constant agitation for 30 min. Several cycles of freeze–thaw extraction followed by placing the sample vials in liquid N_2. Once the bubbling from the liquid N_2 subsided, the vials were subsequently transferred to a 60 °C water bath. Extrusion to a monodispersed suspension of small (e.g., 100-nm diameter) vesicles proceeded by introduction of the polymersome solution into a thermally controlled stainless steel cylinder connected to pressurized nitrogen gas.

For the extraction method, the diblock copolymers organic dilute solution in chloroform or tetrahydrofuran (THF) (about 10mg/ml) was injected into aqueous solution (sucrose solution, PBS or benzene alcohol aqueous solution). Some diblock copolymers particles were extracted from the mixture of organic solvent and water, followed by dialysis at room temperature for 24 hours to remove all organic solvent.

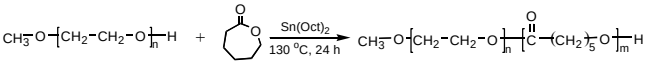
2.5 Characterizations on Morphology of Self-assembly Copolymers

Confocal laser scanning microscopy and a common Zeiss optical microscopy (Axiovert 200) furnished with 488nm laser were employed to characterize the self-assembly copolymers' morphology, respectively. All fluorescence scanning confocal microscope images were obtained by using a Radiance 2000 Multiphoton Confocal System (Bio-Rad) equipped with a 650nm long-pass emission filter (excitation via argon laser, λ_{ex}=488nm).

3 RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Biodegradable PEO-*b*-PCL Diblock Copolymers

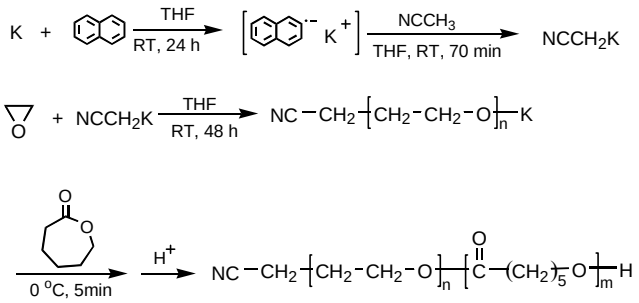
A series of PEO-*b*-PCL diblock copolymers based on commercial MePEO with molecular weights of 5000, 2000, 1100 and 750 were synthesized by ring-opening polymerization at 130°C for 24h (shown in Scheme 1). MePEO with one hydroxyl end group was used as macroinitiator to activate the polymerization of ε-CL monomer under catalysis of stannous(II) octoate ($SnOct_2$).



Scheme 1 synthesis of PEO-*b*-PCL diblock copolymers from MePEO by ring-opening polymerization.

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2

Although it is a simple way to synthesize the copolymer from commercial PEO by ring-opening polymerization of ε-caprolactone monomer, it is impossible to obtain the copolymer with controllable PEO block molecular weight due to limit on the kind of commercial PEO. Anionic living polymerization started from ethylene oxide monomer followed by caprolactone polymerization is another rout to synthesize the copolymers with changeable PEO block molecular weight. Moreover, the end group (terminal) attached to PEO block of the copolymer could be designed by this route. The anionic living polymerization for synthesis of the copolymers with N-terminal group is given in Scheme 2.



Scheme 2 Synthesis of PEO-*b*-PCL diblock copolymers by anionic living polymerization.

Potassium Naphthalenide had been synthesized by ourselves according to the previous reported method. Cynomethyl potassium as initiator for ethylene oxide was prepared by the metalation reaction of acetonitrile with potassium naphthalenide in THF. Only PEO(2.2k)-*b*-PCL(1.2k) with low molecular weight and high PEO weight fraction had previously been synthesized by this anionic living polymerization. We successfully used the revised reaction conditions (Scheme 2 and some description in experimental section) to synthesize PEO-*b*-PCL with a series of PEO block molecular weights (i.e. 1.5k, 2.2k, 2.6k, 3k, 3.8k, and 5.8k), low PEO weight fractions (9.9~23.4%), and a wide molecular weight range (7.8k-47k).

NMR had been proven to be a very useful technique to characterize chemical structure and number-average molecular weight of PEO and PEO-*b*-PCL with different end groups. Appearance of a small peak around 4.20ppm (b') for methylene of PEO block linked to PCL block in the ^{1}H-NMR spectrum of this diblock copolymer demonstrated that the hydrophobic PCL block did connect with the hydrophilic PEO block. A small sharp peak at about 3.38ppm and very strong peak at about 3.65ppm are attributed to methyl (CH_3O- end group of PEO) and methylene groups (repeat unit of MePEO), respectively. Peaks at about 2.23ppm, 1.63ppm, 1.38ppm and 4.06ppm are assigned to protons in PCL repeat units. The peaks used to establish the degree of polymerization for the PCL block and calculate M_n(NMR) are the peaks at 3.65ppm from methylene protons of PEO block and the triplet at 2.23ppm from the methylene protons of caprolactone repeating units in PCL block ($COCH_2CH_2CH_2CH_2CH_2O$). NMR was also used to characterize the number-average molecular weight of PEO from calculated ethylene oxide repeat unit number by calibration of protons from the end group (i.e. CH_3O- or $CNCH_2CH_2$-). The only difference of ^{1}H-NMR spectra between CN-PEO-*b*-PCL diblock copolymers and MePEO-*b*-PCL diblock copolymers is that two weak peaks around 2.50ppm (the first methylene attached to CN, CN-CH_2-CH_2) and 1.90ppm (the second methylene for CN-CH_2-CH_2) for PEO end group of CN-PEO-*b*-PCL replaced the weak peak at 3.38ppm (CH_3O-) for PEO block end group of MePEO-*b*-PCL. Number-average molecular weight values of CN-PEO-*b*-PCL diblock copolymers were also calculated from their NMR spectra as CN-PEO-*b*-PCL diblock copolymers.

GPC is usually employed to characterize molecular weight and polydispersity index (PDI) for molecular weight distribution (M_w/M_n) of polymers. Molecular weight (Mw) and PDI of PCL-*b*-PEO copolymers with different block lengths and ratios were measured by GPC. Two kinds of weight-average molecular weights were calculated from refractive index data by using PEO standard samples and dynamic light scattering data, respectively. Some copolymers, such as PEO(5.8k)-*b*-PCL(24.0k), PEO(5k)-*b*-PCL(22k), PEO(2k)-*b*-PCL(12k), and PEO(2k)-*b*-PCL(15k), exhibited similar molecular weight values from GPC as those from NMR. From GPC data, the copolymers with various PEO molecular weights (2.2k, 2.6k, 3k, 3.8k and 5.8k) synthesized by anionic living polymerization exhibited narrow molecular weight distribution (PDI;1.2-~1.27). The copolymers with PEO (2k) synthesized from ring-opening polymerization showed narrow molecular weight distribution (1.1-1.2) while the copolymers with PEO (5k) displayed slightly wider molecular distribution (PDI:1.32-1.37). Therefore, anionic living polymerization provides a useful route to synthesize the copolymers with controlled PEO block molecular weight, various PEO/PCL block ratios and narrow molecular weight distribution.

3.2 Self-assembly of the Copolymers

Two self-assembly methods, film rehydration and extraction, were chosen to assemble amphiphilic PEO-*b*-PCL diblock copolymers into polymersomes. Because film rehydration is an organic solvent free self-assembly way, all copolymers synthesized by ring-opening polymerization and anionic living polymerization with different molecular weight and block ratios had been subjected to self-assemble by film rehydration.

The perfect polymersomes from PEO(2k)-*b*-PCL(12k) diblock copolymers had been obtained by film rehydration. Some polymersomes with irregular particles could also be prepared from PEO (2k-3.8K)-*b*-PCL(9.5-22.2k) with PEO block weight fractions (f_{PEO}) from 11.8 to 18.8%. Unlike PBD-*b*-PEO diblock copolymers, the range of PEO-*b*-PCL copolymers (f_{PEO}: 11.8-18.8%; PEO block M_n:2k-3.8k; and M_n of the copolymers from 11.5k to 26k) for polymersomes

formation is very narrow. When PEO block has high molecular weight (5 k or 5.8k) or low molecular weight (750-1.5k), no polymersomes was obtained from these copolymers at any ratios of PEO/PCL. From confocal laser fluorescence micrographs in Fig.1, almost 100% polymersomes with sizes from several hundred nanometers to 7-8 microns were made from PEO(2k)-*b*-PCL(12k) after the copolymer's film spontaneously budded off Teflon plate. Many polymersomes have mono-shell structure, but some polymersomes have multi-shell structure. Some big polymersomes contain small polymersomes at their inner space.

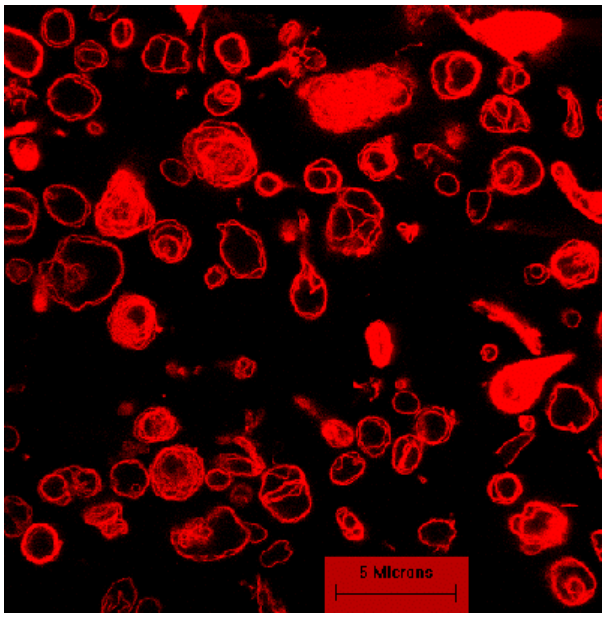


Fig.1 Confocal laser fluorescence micrograph (excitation:488nm) of the micro-polymersomes .

In order to demonstrate if molecular weight distribution has some effect on polymersomes formation, PEO-*b*-PCL containing PEO 2.6k, 3k or 3.8k with very narrow molecular distribution (PDI:1.1), were separated from the copolymers (PDI:1.2) by GPC and used to make polymersomes. However, no any improvement on polymersomes formation from these GPC separated samples was achieved. No polymersomes was made from PEO(5k)-*b*-PCL(22k) with narrow molecular weight distribution (PDI:1.2) separated by GPC. Furthermore, many polymersomes were obtained from mixture of PEO-*b*-PCL copolymers with much wide molecular weight distribution. From above discussion, molecular weight distribution has little influence on biodegradable polymersomes formation from the PEO-*b*-PCL diblock copolymers.

Nanostructured polymersomes with size of 100-200nm could be made by freeze/thaw/extrusion technique. These small polymersomes were characterized by cryo-TEM and the membrane thickness of these polymersomes from PEO(2k)-*b*-PCL(12k) is about 20nm (Fig.2).

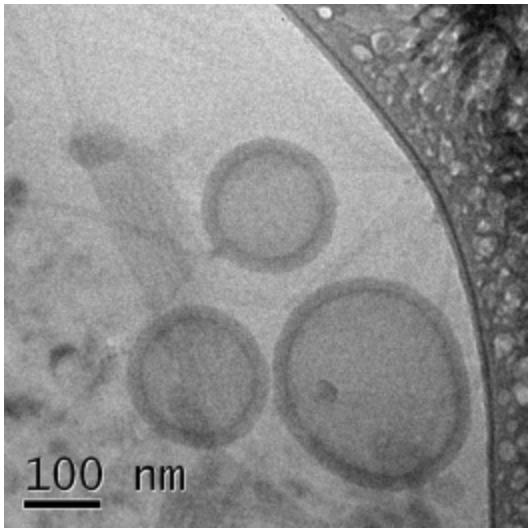


Fig.2 Cryo-TEM micrograph of nanostructured polymersomes.

4 CONCLUSIONS

A series of PEO-*b*-PCL copolymers with PEO blocks (M_n: 750, 1100, 2000 and 5000), PEO weight fractions (7.7%-33.3%) and M_n (3.6k-57k) had been synthesized by ring-opening polymerization of caprolactone monomer using commercial MePEO as initiators. Anionic living polymerization had also been employed to synthesize the PEO-*b*-PCL copolymers with controlled PEO blocks (M_n: 1500, 2200, 2600, 3000, 3800, and 5800), CN as PEO block terminal groups, f_{PEO} (9.9-23.4%) and M_n of the copolymers (7.8k-47k). All copolymers have molecular weight distribution (polydiserpsity index, PDI) from 1.14 to 1.37. These copolymers had been used to self-assemble into polymersomes by film rehydration and extraction. The perfect polymersomes were obtained from PEO(2k)-*b*-PCL(12k) (PDI:1.21) by film rehydration. The range of these copolymers for polymeromes formation is PEO (2k-3.8k) and f_{PEO} (11.8-18.8%). Molecular weight distribution has little influence on the biodegradable polymersomes formation. The film rehydration is a better self-assembly method for preparing the biodegradable polymersomes than the extraction method.

REFERENCES

[1] D.E. Discher, A. Eisenberg, Science, 297, 967-973, 2002.

[2] B.M. Discher, Y.Y. Won, D.S. Ege, J.C.M. Lee, F.S. Bates, D.E. Discher, D.A. Hammer, Science 284, 1143-1146, 1999.

[3] P.P Ghoroghchian, P.R. Frail, K. Susumu, D. Blessington, A.K. Brannan, F.S. Bates, B. Chance, D.A. Hammer, M.J. Therien, PNAS, 102, 2922-2927, 2005.

[4] P. Ghoroghchian, G. Li, K.P. Davis, F.S. Bates, D.A. Hammer, M.J. Therien, Macromolecules, 39(5), 1673-75, 2006.