J. Soc. Cosmet. Sci. Korea
Vol. 43, No. 2, June 2017, 165-174

pISSN 1226-2587
eISSN 2288-9507
http://dx.doi.org/10.15230/SCSK.2017.43.2.165

Poly (lactide)-*b*-Poly (glycerol) 블록 공중합체의 중합

이 존 환·오 성 근·김 용 진*†

한양대학교, *아모레퍼시픽

(2017년 5월 29일 접수, 2017년 6월 29일 수정, 2017년 6월 30일 채택)

Synthesis of Poly (lactide)-*b*-Poly (glycerol) (PLA-*b*-PG) Block Copolymer

John Hwan Lee, Seong-Geun Oh, and Yong-Jin Kim*†

Department of Chemical Engineering, Hanyang University, 222, Wangsimni-ro, Seongdong-gu, Seoul 04763, Korea
*R&D Unit, AmorePacific Corporation, 1920, Yonggu-daero, Giheung-gu, Yongin-si, Gyeonggi-do 17074, Korea

(Received May 29, 2017; Revised June 29, 2017; Accepted June 30, 2017)

요 약: 이 연구는 소수성 폴리락타이드(PLA) 블록과 친수성 하이퍼브랜치드 폴리글리세롤(*hb*PG) 블록으로 구성된 양친매성 블록 공중합체(PLA-*b*-*hb*PG)의 합성방법에 대한 것이다. 또한, *hb*PG 블록을 4-hydroxyl cinnamic acid (CA)로 에스터 반응화하여, 광가교가 가능한 블록 공중합체인 PLA-*b*-*hb*PG-CA에 대한 접근법에 대해서도 보고하였다. 연구된 양친성 고분자는 친수기에 많은 양으로 존재하는 폴리글리세롤에 의해 화장품용 약물 전달체로 사용이 가능한 작은 크기(100 nm)의 마이셀을 형성함을 확인하였다. 또한, *hb*PG으로 구성된 마이셀의 corona 부분은 우수한 친수성을 나타내어 생체 내 독성을 최소화할 수 있음을 확인하였다. 소수성 활성성분이 담지된 PLA-*b*-*hb*PG-CA 마이셀은 생체적합성 및 자기조립구조에 의해 화장품용 약물 전달체로 활용이 가능할 것으로 기대된다.

Abstract: This study reports a synthesis of an amphiphilic linear block copolymer consisting of a hydrophobic poly (lactide) (PLA) block and a hydrophilic hyperbranched polyglycerol (*hb*PG) block, PLA-*b*-*hb*PG. Simple chemical modification of the *hb*PG block with 4-hydroxycinnamic acid (CA) led to a photo-crosslinkable block copolymer, PLA-*b*-*hb*PG-CA. Nanosized micelles of the block copolyemrs were used as drug carriers for sustainable release. The *hb*PG shell made of a small molecular weight *hb*PG block showed excellent hydrophilicity, which can minimize *in vivo* toxicity. The UV-crosslinked PLA-*b*-*hb*PG-CA micelles loaded with drugs colud be served as a drug delivery carrier for its biocompatibility and self-assembled structures.

Keywords: *hyperbranched polyglycerol, polymer synthesis, sustained release, amphiphilic block copolymer, drug delivery systems*

1. Introduction

Synthetic biocompatible, amphiphilic block copolymers have been widely used as drug delivery carriers because their chemical composition and architecture are easily modified to regulate the release of drugs from carriers. The carriers are typically composed of a drug-loading cargo and an environment-compatible periphery, such as polymersomes[1], dendritic particles[2], nanogels[3], and micelles[4]. Although tremendous efforts have been devoted to the use of the polymer-based formulations for clinical applications, only a few systems have advanced to the

† 주 저자 (e-mail: jaykim714@gmail.com)
call: 031)280-5873

clinical settings due to toxicity of polymers, arising mainly from the non-biodegradable hydrophilic part of carriers. Highly biocompatible, hydrophilic polymers such as poly (ethylene oxide)[5], polyglycerols[6], and sugar derivatives[7] have been employed to decrease the toxicity. Among them, polyglycerols, a class of polyethers with a hydroxy graft in each segment, have been of great interest due to their excellent biocompatibility[8-12]. Diverse architectures of polyglycerols have been designed and employed for drug delivery[10], protein delivery[11], and gene transfection[12]. The easy generation of hyperbranches using their hydroxyl groups have attracted specific interest[13]. Hyperbranched polyglycerol (*hb*PG) shell can provide enhanced excellent biocompatibility and long-term *in vivo* stability for drug carriers even when the molecular weight of *hb*PG is very small, which will minimize the potential toxicity *in vivo*[14]. Surprisingly, to the best of our knowledge, all the *hb*PG-based block copolymers reported so far have consisted of non-biodegradable hydrophobic blocks such as polypropylene glycol[15] and poly (ethoxyethyl glycidyl ether) (PEEGE)[16]. The carriers with the non-biodegradable cores can have drawbacks in the practical applications involving cellular uptake.

Sustained release of drugs has been an important issue in drug delivery systems. Crosslinking the shell of a micelle triggered by external light has been actively explored due to their convenience for remote control and their potential of high spatiotemporal resolution[17-21].

Kim et al. investigated the shell cross-linked micelles composed of Tetronic-tyramine and heparin-tyramine, which showed the sustained release behavior of indomethacin and basic fibroblast growth factors compared with non-cross-linked micelles[22]. Xuet al. reported another shell cross-linked micelle for sustained release of doxorubicin via self-cross linking of lipoic acid in the polyethylene glycol-lipoic acid-polylactide[23]. Yuan et al. reported the controlled release of a fluorescent dye from photo-responsive nitrocinnamate capsules through reversible photo-crosslinking/photo-cleavage under UV irradation[24].

In this study, we synthesized, for the first time, an amphiphilic block copolymer consisting of poly (lactide)-*block*-hyperbranched polyglycerols (PLA-*b*-*hb*PG), which can self-assemble into micelles in the presence of hydrophobic therapeutic drugs. We incorporated cinnamic acid (CA) in the *hb*PG block for photo-crosslinking of the shell layer of PLA-*b*-*hb*PG micelles, which led to sustained release of a hydrophobic drug, 4-n-butylresorcinol (BR).

2. Experimental

2.1. Materials

N,N'-dimethylformamide (DMF, anhydrous 99.8%), dimethylsulfoxide (DMSO, anhydrous 99.9%), benzene (99.8%), N,N'-dicyclohexylcarbodiimide (99%), 4-(dimethylamino)pyridine (99%), glycidol (96%), ethyl vinyl ether, p-toluenesulfonic acid monohydrate (TsOH, 98.5%), glycerol (99.5%), ethyl vinyl ether (98%), aluminium chloride hexahydrate (99%), lactide (97%), stannous octanoate ($Sn(Oct)_2$, 95%), trans-cinnamic acid (99%), dichloromethane (anhydrous 99.8%), diethylene glycol dimethyl ether (diglyme, anhydrous 99.9%), and potassium *t*-butoxide (98%) were purchased from Sigma. For polymerization, diglyme and glycerol were purified by distillation from CaH_2 directly prior to use. The other reagents and solvents were used as received.

2.2. Characterization

^1H and ^{13}C NMR spectra of polymers were taken (300 MHz, Varian) in DMSO as a solvent. Size exclusion chromatography (SEC) was conducted with DMF solution (containing 1 g/L of lithium bromide as an additive). The solution passed through the PSS Gral column (104/104/102 Å porosity) and the polymer chains were monitored with a RI detector. Standard polystyrenes with different molecular weights were used for calibration of molecular weight. Transmission electron microscopy (TEM, JEM-2100, JEOL) was used to investigate the morphology of PLA-*b*-*hb*PG micelles. Size of the micelles were measured with dynamic light scattering (DLS, Nano

ZS, UK).

2.3. Synthesis of Ethoxyethyl Glycerol Ether (EEGE)

This compound was prepared following the method described in the previous literature[25]. Briefly, 85.0 g (1.147 mol) of glycidol and 225.9 g (3.133 mol) of ethyl vinyl ether were introduced in a 500 mL two-neck flask. After magnetic stirring for 3 min, the mixture solution was cooled to -30 ℃. 1.915 g (11.12 mmol) of *p*-toluenesulfonic acid monohydrate (TsOH), which is 1.0 mol % to glycerol, was slowly added to the cold mixture solution, followed by magnetic stirring of the mixture solution at 25 ℃. After 3 h, the mixture solution was then washed with a saturated aqueous $NaHCO_3$ solution. The organic layer was separated and dried with $MgSO_4$. After filtration, the residual ethyl vinyl ether was removed at reduced pressure (0.6 Torr) and a raised temperature (50 ℃). The remainder was distilled in vacuum to yield EEGE as a colorless liquid product (yield = 74%).

2.4. Polymerization of Ethoxyethyl Glycidyl Ether (PEEGE)

We modified the procedure described in the literature[26]. A 100 mL round-bottom flask was dried by heating under a stream of nitrogen. A potassium *t*-butoxide solution in anhydrous THF (2.25 mL, 1 M) was introduced in the dried flask under nitrogen environment. The reactor was cooled to -50 ℃ and EEGE (10 mL) was added. The temperature was slowly increased to 60 ℃ and then maintained the same during the polymerization process. After 17 h polymerization, cold methanol of 10 ℃ was added to the reactor and the reaction was kept for 2 h at room temperature. The solvent was evaporated at 30 ℃. The resulting polymer was dissolved in benzene and centrifuged to remove the precipitated potassium chloride, followed by drying in vacuum for 24 h.

2.4. Synthesis of PLA–*b*–PEEGE

The linear block copolymer was prepared by the modified ring opening polymerization of lactide[27]. Briefly, 1 g (0.24 mmol) of PEEGE (M_n = 4,100 g/mol, determined by GPC) and 0.5 g of lactide (4.38 mmol) were introduced in a 50 mL round-bottom flask containing a Teflon-coated magnetic stirring bar. The flask was degassed in vacuum and purged with argon gas flowing through a rubber septum. The mixture was melted in an oil bath pre-heated at 120 ℃. Immediately, stannous octanoate (0.015 mmol) was introduced into the flask through the septum *via* gastight syringes. The reaction was conducted for 20 h at the same temperature, and the flask was cooled to room temperature. The resulting polymer was dissolved in 20 mL of methylene chloride and then precipitated into a 10-fold excess of cold hexanes.

2.5. Preparation of PLA–*b*–PG

The procedure is similar to a method reported recently for deprotection of tetrahydropyranyl ethers[28]. Given amount of PLA-*b*-PEEGE was dissolved in MeOH, followed by addition of $AlCl_3$-$6H_2O$. The reaction was conducted for 0.5 h at room temperature. The molar ratio was [-EEGE-] : $AlCl_3$: MeOH = 100 : 1 : 800. The reaction product was filtered through diatomaceous earth, and the solvents were evaporated under low pressure.

2.6. Synthesis of Hyperbranched (*hb*) Block Copolymer PLA–*b*–*hb*PG

Hypergrafting of the linear block copolymer PLA-*b*-PG was performed by using the Wurm's method[29]. The linear macro-initiator PLA-*b*-PG (1 g, 3.35 mmol as OH function) was placed in a two-neck flask and dissolved in benzene (3.5 mL, ca. 20 wt%). Potassium *t*-butoxide (0.09 g, 0.805 mmol) was added to deprotect 25% of the hydroxyl groups along the backbone of polyglycerol block in PLA-*b*-PG. The heterogeneous solution was stirred at 60 ℃ for 30 min, and the solvents were removed with a rotary evaporator. The opaque viscous liquid was heated at 90 ℃ and dried under low pressure for 1 h. After nitrogen was introduced into this flask, 3.5 mL of anhydrous diglyme was added (ca. 20 wt%). The flask was placed in an ultrasonic bath for 30 min, and the mixture was heated to 90 ℃. Then, glycerol (0.3 g, 4 mmol) in 8 mL of diglyme was slowly added with a syringe over a period of approximately 24 h. The reaction was termi-

nated by addition of excess methanol containing an acidic cation exchange resin (Dowex® 50WX8). This mixture was filtered and poured into cold diethyl ether; the precipitate was dried at 40 ℃ for 2 days to obtain the hyper-grafted PLA-*b*-*hb*PG copolymer with a yield of 0.91 g (68%).

2.7. Preparation of Cinnamic Acid (CA)–terminated Block Copolymer PLA–*b*–*hb*PG–CA

PLA-*b*-*hb*PG-CA was prepared by coupling reaction with N,N'-dicyclohexyl carbodiimide (DCC). 110 mg DMAP and 0.4 mmol PLA-*b*-*hb*PG were added to a solution of 2.9 mmol trans-cinnamic acid in 20 mL anhydrous DMF under gentle stirring. 1.2 g of DCC was added to the reaction mixture at room temperature, and the solution was stirred at 0 ℃ for 5 min and then at 20 ℃ for 3 h. Precipitated urea was filtered through Whatman No. 2 filter paper. The solvent in the filtrate was evaporated under vacuum and the residue was collected by dissolving in diethyl ether.

2.8. Cytotoxicity Test of PLA–*b*–*hb*PG–CA

50 mL of 2 mg/mL MTT dissolved in Minimal Essential Media (MEM) or Dulbecco's Modified Eagle's Medium was added to HaCaT keratinocyte cells and incubated in a humidified atmosphere of 5% CO_2 at 37 ℃. After 12 h, the growth medium was replaced with 200 μL of complete DMEM culture medium that contained the desired amount of samples solved in dimethyl sulfoxide. The cells treated with the same amount of PBS were used as a control group. The cells were incubated for another 48 h and the cell viability was assayed by adding 20 μL of MTT assay (Sigma) PBS solution (5 mg/mL). The viability of cells was determined by measuring the absorbance at a test wavelength of 540 nm (MRX-microplate reader).

2.9. Preparation of PLA–*b*–*hb*PG–CA Micelle Encapsulating 4–n–butylresorcinol (BR)

PLA-*b*-*hb*PG-CA micelles encapsulated with BR were prepared by using precipitation method. 0.2 g of BR and 2 g of PLA-*b*-*hb*PG-CA were dissolved in 50 mL of MeOH. The solution was poured into 50 mL of deionized (DI) water with vigorous stirring. Then, the mixture was moved to a rotary evaporator to remove MeOH. After evaporation for 1 h at 40 ℃, the PLA-*b*-*hb*PG-CA micelles encapsulated with BR were filtrated by 0.45 μm syringe filter. The micelles were washed three times with fresh water to remove the residual methanol.

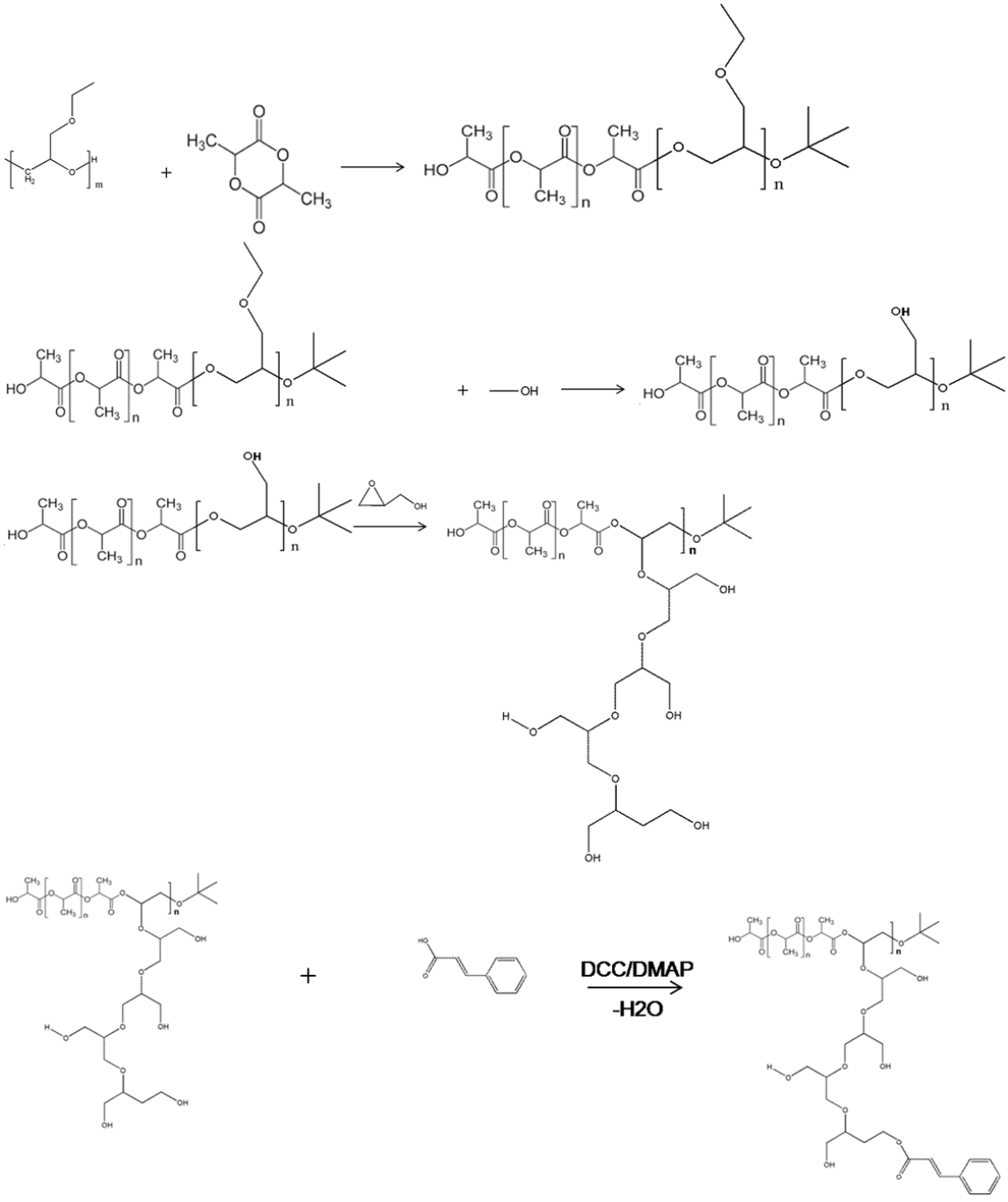
3. Results and Discussions

3.1. Analysis of the Synthesized PLA–*b*–*hb*PG–CA Block Copolymers

The synthetic route to PLA-*b*-*hb*PG is presented in **Scheme 1**. Poly (ethoxy ethyl glycidyl ether) (PEEGE) was synthesized by anionic polymerization of EEGE initiated with potassium *t*-butoxide, as established by Dworak *et al*.[26] The terminal hydroxy group of PEEGE was used to initiate a ring opening polymerization of lactide in the presence of $Sn(Oct)_2$, synthesizing PLA-*b*-PEEGE block copolymer. The protecting acetal group in PEEGE block was cleaved by methanol in the presence of $AlCl_3$, which produced PLA-*b*-PG. The deprotection yield was 95%.

The PLA-*b*-PG was used as a macroinitiator to initiate the hyperbraching reaction. Commonly, an acid solvent including HCl was known to cleave the protecting ethoxy ethyl group in PEEGE block copolymers[28]. To obtain polymer with high molecular weight, the deprotection reaction had to proceed under non-acidic condition because PLA block was easily attacked under acidic condition[30]. The use of $AlCl_3$ as a catalyst for deprotection reaction prevented the hydrolytic degradation of ester group in PLA[28], which allowed for the formation of a series of high molecular weight PLA blocks.

Slow addition of glycerol monomer to the linear PLA-*b*-PG enabled conversion of the linear PG block into the hyperbranched PG block (*hb*PG), leading to the generation of PLA-*b*-*hb*PG block copolymer[31]. In the reaction, 10% of terminal OH groups of the linear PG block were deprotonated by potassium tert-butoxide,



Scheme 1. Synthetic route to poly (lactide)-*b*-hyperbranched poly (glycerol) (PLA-*b*-*hb*PG) and attachment of cinnamic acid (CA) to the *hb*PG block.

and the formed oxoanion groups acted as a macroinitiator for anionic ring opening reaction of the epoxy group in the glycidol monomer. To prerpare sustained delivery by crosslinking the shell of the micelles, cinnamic acid (CA) was reacted with glycerol groups in the *hb*PG block through the coupling reaction with N,N'-dicyclohexylcarbodiimide (DCC)[32].

Number-average molecular weights (M_n) of the poly-

Table 1. Number-average molecular weights (M_n) and polydispersity indexes (PDIs) of the polymers synthesized in this study; polyethoxy ethyl glycidyl ether (PEEGE), poly (lactide)-*b*-poly (glycerol) (PLA-*b*-PG), and poly (lactide)-*b*-hyperbranched poly (glycerol) (PLA-*b*-*hb*PG)

Sample	M_n (GPC) (g/mol)	PDI (M_w/M_n)	Yield (%)
PEEGE	4,100	1.13	91%
PLA-*b*-PG	4,180	1.26	94%
PLA-*b*-*hb*PG	4,860	1.29	81%

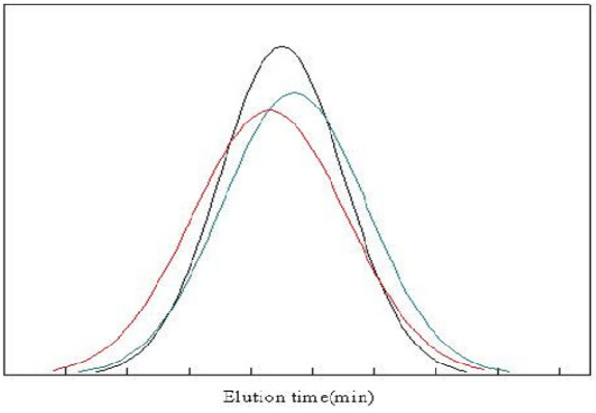


Figure 1. (A) GPC traces of the polymers synthesized in this study; PEEGE, PLA-*b*-PG, and PLA-*b*-*hb*PG (black line; PEEGE, red line; PLA-*b*-*hb*PG, green line; PLA-*b*-PG).

mers synthesized in this study were determined with GPC (Figure 1A). DMF was used as the solvent. The measured M_n values are summarized in Table 1. M_n of PEEGE was 4,100 g/mol and polydispersity index (PDI, M_w/M_n) was 1.13. M_n of the block copolymer (PLA-*b*-PG) was 4,180 g/mol (M_w/M_n = 1.26), which corresponds to 94% deprotection yield. M_n of PLA block in the PLA-*b*-PG was 2,088 g/mol; hence, its degree of polymerization was 18. M_n of the hyperbranched block copolymer (PLA-*b*-*hb*PG) increased slightly (M_n = 4,860) by the amount of hyperbranches. From the molecular weight change, the average number of glycerol monomers transformed into the hyperbranched repeating units was calculated to be 0.3. After the reaction with cinnamic acid, the molecular weight of PLA-*b*-*hb*PG-CA increased to 5,648 which corresponds to 97% coupling reaction yield (Data are not shown here).

The chemical structures of the PLA-*b*-*hb*PG and

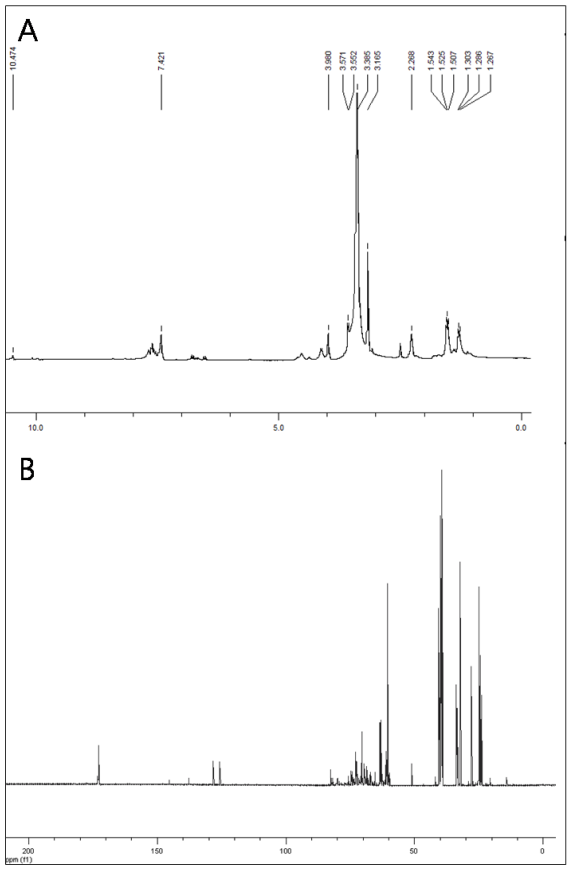


Figure 2. ^{1}H NMR spectra of PLA-*b*-*hb*PG-CA (A) and ^{13}C NMR of PLA-*b*-*hb*PG-CA (B) in DMSO-*d*6.

PLA-*b*-*hb*PG-CA were investigated with NMR (^{1}H, ^{13}C) (Figure 2). The NMR result showed that the PLA-*b*-*hb*PG block copolymer had a well-defined hyperbranched PG structure (Figure 2A). The peaks in ^{1}H NMR (400 MHz, DMSO-d6) are assigned as followings: δ 1.10 (t, 3H, -CH_3), δ 1.19 (q, 2H, -OCH_2CH_3), δ 2.53-2.73 (m, 2H, CH_2 of the epoxy ring), δ 3.07-3.09 (m, 1H, CH of the epoxy ring), δ 3.25-3.77 (m, 4H, -OCH_2CH_3 and

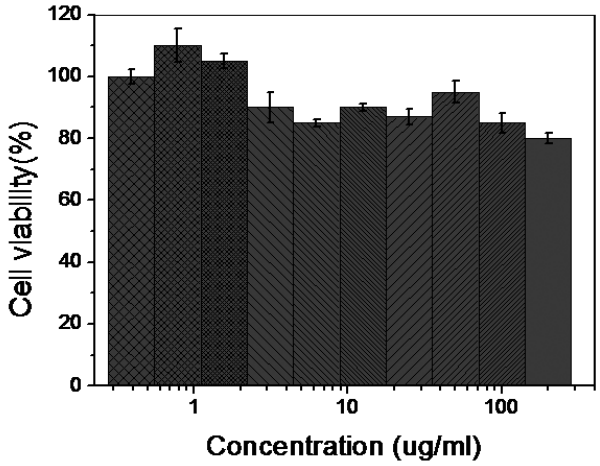


Figure 3. Cell viability of PLA-*b*-*hb*PG-CA by MTT assay.

-OCH_2-), and δ 4.69 (m, -OCH (CH_3) O-) ppm. The peaks in ^{13}C NMR (101 MHz, DMSO-d6) appeared at δ 15.2, 19.8, 39.5, 43.6, 50.4, 60.3, and 66.0 ppm (See Figure 2B). The average number of glycerol monomers in the hyperbranched repeating unit was calculated with GPC and the value was 34. As shown in Figure 2A, ^1H NMR specturm of PLA-*b*-*hb*PG-CA showed peaks at 6.8 and 8.0 ppm, which were assigned to the carbon double bonds of the cinnamate groups. This result indicates that the CA reacted successfully with the hydroxy groups of the *hb*PG block.

The PLA-*b*-*hb*PG-CA block copolymer can have great potential as a drug carrier due to its excellent biocompatibility and chemical versatility, arising from the abundant hydroxy groups. We tested the *in vitro* cytotoxicity of the PLA-*b*-*hb*PG-CA micelles based on the viability of HaCaT keratinocyte cells (Figure 3). The cell viablities were above 80% at all concentrations, indicating that the PLA-*b*-*hb*PG-CA micelles were non-toxic and had good biocompatibility.

3.2. Characterization of the Drug-loaded Micelles

4-n-butylresorcinol-loaded micelles made of the PLA-*b*-*hb*PG-CA were prepared by precipitation method. The loading efficiency defined as a weight percent of loaded drug, relative to initial drug used for the micelle formation, was 95%. The shape and size of the micelles

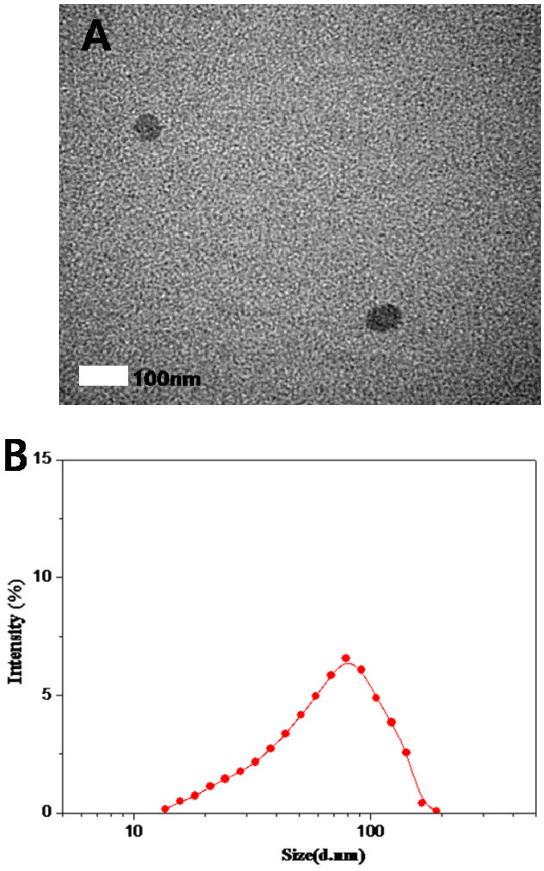


Figure 4. (A) TEM images of the PLA-*b*-*hb*PG polymeric micelles encapsulating 4-n-butylresorcinol (BR), (B) dynamic light scattering (DLS) analysis of the PLA-*b*-*hb*PG-CA. Polymeric micelles encapsulating 4-n-butylresorcinol.

were characterized with transmission electron microscopy (TEM) and dynamic light scattering (DLS) (Figure 4). Figure 4 shows TEM images of the BR-loaded PLA-*b*-*hb*PG-CA micelles, demonstrating that the monodispersed, nanosized micelles were fabricated. The mean diameter of the micelles was measured to be 100 nm by DLS as shown in Fgure 4B. This narrow size distribution is attributed to the enhanced hydration, originating from a large number of glycerol groups in the outter shell. The low interfacial tension between the micelles and water could effectively stabilize the micelles in water, thereby leading to the decrease and good uniformity in the size of the micelles. It is noticible that both the micelles did not show any precipitation over 2 weeks,

which suggests that introducion of a relatively small amount of cinnamic acid groups did not cause destabilization of the micelles.

4. Conclusions

A novel amphiphilic diblock copolymer (PLA-*b*-*hb*PG) consisting of a biodegradable poly (lactide) (PLA) block and a hyperbranched polyglycerol (*hb*PG) block. By attaching 4-hydroxycinnamic acid (CA) to the *hb*PG block, that is PLA-*b*-*hb*PG-CA, the hydrophilc block could be crosslinked by UV irradiation. The high hydrophilicity of *hb*PG block gave an amphiphilic property to the block copolymer. The low molecular weight of *hb*PG is expected to decrease possible *in vivo* toxicity. The block copolymers formed spherical nanoscale micelles in the presence of a hydrophobic drug, 4-n-butylresorcinol. The shell of the PLA-*b*-*hb*PG-CA micelles could be cross-linked by UV irradiation.

Reference

1. F. Ahmed, R. I. Pakunlu, A. Brannan, F. Bates, T. Minko, and D. E. Discher, Biodegradable polymersomes loaded with both paclitaxel and doxorubicin permeate and shrink tumors, inducing apoptosis in proportion to accumulated drug, *J. Control. Release*, **116**, 150 (2006).
2. J. Khandare, M. Calderón, N. Dagia, and R. Haag, Multifunctional dendritic polymers in nanomedicine: opportunities and challenges, *Chem. Soc. Rev.*, **41**, 2824 (2012).
3. A. V. Kabanov and S. V. Vinogradov, Nanogels as pharmaceutical carriers: finite networks of infinite capabilities, *Angew. Chem. Int. Ed.*, **48**, 5418 (2009).
4. F. Yhaya, J. Lim, Y. Kim, M. Liang, A. M. Gregory, and M. H. Stenzel, Development of micellar novel drug carrier utilizing temperature-sensitive block copolymers containing cyclodextrin moieties, *Macromolecules*, **44**, 8433 (2011).
5. J. P. Jain and N. Kumar, Self-assembly of amphiphilic (PEG) 3-PLA copolymer as polymersomes: preparation, characterization, and their evaluation as drug carrier, *Biomacromolecules*, **11**, 1027 (2010).
6. X. Zhang, K. Achazi, D. Steinhilber, F. Kratz, J. Dernedde, and R. Haag, A facile approach for dual-responsive prodrug nanogels based on dendritic polyglycerols with minimal leaching, *J. Control. Release*, **174**, 209 (2014).
7. J. C. Hooton, M. D. Jones, and R. Price, Predicting the behavior of novel sugar carriers for dry powder inhaler formulations via the use of a cohesive-adhesive force balance approach, *J. Pharm. Sci.*, **95**, 1288 (2006).
8. S. Lee, K. Saito, H. Lee, M. Lee, Y. Shibasaki, Y. Oishi, and B. Kim, Hyperbranched double hydrophilic block copolymer micelles of poly (ethylene oxide) and polyglycerol for pH-responsive drug delivery, *Biomacromolecules*, **13**, 1190 (2012).
9. A. Zarrabi, M. A. Shokrgozar, M. Vossoughi, and M. Farokhi, *In vitro* biocompatibility evaluations of hyperbranched polyglycerol hybrid nanostructure as a candidate for nanomedicine applications, *J. Mater. Sci. - Mater. Med.*, **25**, 499 (2014).
10. M. Hu, M., Chen, M., G. Li, Y. Pang, Y. Wang, J. Wu, F. Qiu, X. Zhu, and J. Sun, Biodegradable hyperbranched polyglycerol with ester linkages for drug delivery, *Biomacromolecules*, **13**, 3552 (2012).
11. D. Steinhilber, M. Witting, X. Zhang, M. Staegemann, F. Paulus, W. Friess, S. Küchler, and R. Haag, Surfactant free preparation of biodegradable dendritic polyglycerol nanogels by inverse nanoprecipitation for encapsulation and release of pharmaceutical biomacromolecules, *J. Control. Release*, **169**, 289 (2013).
12. A. Tschiche, A. M. Staedtler, S. Malhotra, H. Bauer, C. Böttcher, C. Sharbati, M. Calderón, M. Koch, T. M. Zollner, A. Barnard, D. K. Smith, R. Einspanier, N. Schmidt, and R. Haag, Polyglycerol-based amphiphilic dendrons as potential siRNA carriers for in vivo applications, *J. Mater. Chem. B*, **2**, 2153 (2014).
13. D. Wilms, S. E. Stiriba, and H. Frey, Hyperbranched

polyglycerols: from the controlled synthesis of biocompatible polyether polyols to multipurpose applications, *Acc. Chem. Res.*, **43**, 129 (2010).
14. K. Kainthana and D. E. Brooks, *In vivo* biological evaluation of high molecular weight hyperbranched polyglycerols, *Biomaterials*, **28**, 4779 (2007).
15. F. Wurm and H. Frey, Linear-dendritic block copolymers: the state of the art and exciting perspectives, *Prog. Polym. Sci.*, **36**, 1 (2011).
16. Y. Oikawa, S. Lee, D. Kim, D. Kang, B. Kim, K. Saito, S. Sasaki, Y. Oishi, and Y. Shibasaki, One-pot synthesis of linear-hyperbranched amphiphilic block copolymers based on polyglycerol derivatives and their micelles, *Biomacromolecules*, **14**, 2171 (2013).
17. X. Li, H. Wen, H. Dong, W. Xue, G. Pauletti, X. Cai, W. Xia, D. Shi, and Y. Li, Self-assembling nanomicelles of a novel camptothecin prodrug engineered with a redox-responsive release mechanism, *Chem. Commun.*, **47**, 8647 (2011).
18. W. Chen, P. Zhong, F. Meng, R. Cheng, C. Deng, J. Feijen, and Z. Zhong, Redox and pH-responsive degradable micelles for dually activated intracellular anticancer drug release, *J. Control. Release*, **169**, 171 (2013).
19. D. Shi, M. Matsusaki, T. Kaneko, and M. Akashi, Photo-cross-linking and cleavage induced reversible size change of bio-based nanoparticles, *Macromolecules*, **41**, 8167 (2008).
20. Z. G. Gao, A. N. Lukyanov, A. Singhal, and V. P. Torchilin, Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs, *Nano Lett.*, **2**, 979 (2002).
21. Y. Matsumura and H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs, *Cancer Res.*, **46**, 6387 (1986).
22. B. Kim, J. Bae, and K. Park, Enzymatically in situ shell cross-linked micelles composed of 4-arm PPO-PEO and heparin for controlled dual drug delivery, *J. Control. Release*, **172**, 535 (2013).
23. Y. Xu, F. Meng, R. Cheng, and Z. Zhong, Reduction-sensitive reversibly crosslinked biodegradable micelles for triggered release of Doxorubicin, *Macromol. Biosci.*, **9**, 1254 (2009).
24. W. Yuan, K. Fischer, and K. Schärtl, Photocleavable microcapsules built from photoreactive nanospheres, *Langmuir*, **21**, 9374 (2005).
25. A. Fitton, J. Hill, D. E. Jane, and R. Millar, Synthesis of simple oxetanes carrying reactive 2-substituents, *Synthesis*, **12**, 1140 (1987).
26. A. Dworak, L. Panchev, B. Trzebicka, and W. Walach, Poly (α-t-butoxy-ω-styrylo-glycidol): a new reactive surfactant, *Polym. Bull.*, **40**, 461 (1998).
27. F. Ahmed and D. E. Dische, Self-porating polymersomes of PEG-PLA and PEG-PCL: hydrolysis-triggered controlled release vesicles, *J. Control. Release*, **96**, 37 (2004).
28. P. Dimitrova, A. Porjazoskab, C. P. Novakova, M. Cvetkovskab, and C. B. Tsvetanov, Functionalized micelles from new ABC polyglycidol-poly (ethylene oxide)-poly (d, l-lactide) terpolymers, *Polymer*, **46**, 6820 (2005).
29. F. Wurm, J. Nieberle, and H. Frey, Double-hydrophilic linear-hyperbranched block copolymers based on poly (ethylene oxide) and poly (glycerol), *Macromolecules*, **41**, 1184 (2008).
30. M. Gervais, A. Brocas, G. Cendejas, A. Deffieux, and S. Carlott, Synthesis of linear high molar mass glycidol-based polymers by monomer-activated anionic polymerization, *Macromolecules*, **43**, 1778 (2010).
31. A. Sunder, R. Hanselmann, H. Frey, and R. Mülhaupt, Controlled synthesis of hyperbranched polyglycerols by ring-opening multibranching polymerization, *Macromolecules*, **32**, 4240 (1999).
32. B. Neises and W. Steglich, Simple method for the esterification of carboxylic acids, *Angew. Chem. Int. Ed.*, **17**, 522 (1978).
33. A. Khemis, A. Kaiafa, C. Queille-Roussel, L. Duteil, and J. P. Ortonne, Evaluation of efficacy and safety of rucinol serum in patients with melasma: a randomized controlled trial, *Br. J. Dermatol.*, **156**, 997 (2007).

34. J. Kim, J. Shim, Y. Kim, K. Char, K. Suh, and J. Kim, The design of polymer-based nanocarriers for effective transdermal delivery, *Macromol. Biosci.,* **10**, 1171 (2010).