Biodegradable Amphiphilic Triblock Copolymer Bearing Pendant Glucose Residues: Preparation and Specific Interaction with Concanavalin A Molecules

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Received February 9, 2006; Revised Manuscript Received April 10, 2006

A novel biodegradable amphiphilic block copolymer PLGG-PEG-PLGG bearing pendant glucose residues is successfully prepared by the coupling reaction of 3-(2-aminoethylthio)propyl-α-D-glucopyranoside with the pendant carboxyl groups of PLGG-PEG-PLGG in the presence of *N*,*N*′-carbonyldiimidazole. The polymer PLGG-PEG-PLGG, i.e., poly{(lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]}-*block*-poly(ethylene glycol)-*block*- poly-{(lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]}, is prepared by ring-opening copolymerization of L-lactide (LLA) with (3*s*)-benzoxylcarbonylethylmorpholine-2,5-dione (BEMD) in the presence of dihydroxyl PEG with molecular weight of 2000 as macroinitiator and $Sn(Oct)_2$ as catalyst, and then by catalytic hydrogenation. The glucose-grafted copolymer shows a lower degree of cytotoxicity to ECV-304 cells and improved specific recognition and binding with Concanavalin A (Con A). Therefore, this kind of glucose-grafted copolymer may find biomedical applications.

Introduction

Polylactide (PLA), an important kind of synthetic biodegradable polymer, has been widely used in biomedical applications in recent years, such as drug delivery and tissue engineering.[1−6] Although PLA has many good properties, such as biodegradability, biocompatibility, high mechanical strength, and so forth, its application in some fields is still limited because of its hydrophobicity and lack of functional or reactive pendant groups along the molecular chain. If the molecular chains of PLA can carry reactive pendant groups, it is possible to chemically attach drugs or other bioactive molecules so as to facilitate more biomedical applications of the polymer.[7−9] Therefore, the preparation of polylactide containing pendant functional groups has attracted much attention in the past few years.[10−17]

Poly(ethylene glycol) (PEG), with characteristics of nontoxicity, hydrophilicity, and biocompatibility, is often used to synthesize amphiphilic block copolymers with PLA which have better properties compared to PLA, such as good hydrophilicity and self-assembling ability. Currently, many studies focus on this kind of copolymer in expectation of achieving wide applications.[18−21]

As well-documented in the literature, sugars play a critical role in the process of cell recognition.[22,23] Carbohydrate-substituted polymers have been used to recognize some proteins on the basis of specific protein−saccharide interactions.[23−28] Since Roy and Horejsl first reported the glycopolymers prepared from allyl glycosides and acrylamide,[29] many kinds of glycoconjugates have been synthesized using analogous alkene-terminated glycosides.[30] However, the glycopolymers with carbon−carbon bonds in the main chain are not biodegradable. Recently, diverse sugar (glucose, manose, galactose, and lactose)-capped poly(ethylene glycol)-*b*-poly(D,L-lactide) block copolymers are synthesized, and their micelles of core−shell structure are obtained from water media.[31−33] With the probe sugars on their surface, the micelles have a specific binding ability to target cells.[34] Therefore, this kind of polymeric micelle systems would be a cell-targeting drug carrier. However, an A−B type of block copolymer end-capped with only one sugar residue is less effective than a carbohydrate-grafted polymer in mimicking the multivalent carbohydrate−protein interaction.[35] On the other hand, the carbohydrate-grafted degradable polymers are also important models for the study of the interactions between multivalent ligands and the target specific receptors. However, there are no reports on carbohydrate-grafted polyesters, such as PLLA.

In the present study, a novel triblock copolymer P(LGG/glucose)-PEG-P(LGG/glucose) which is derived from its parent polymer P(LGG)-PEG-P(LGG), i.e., poly{(lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]}-*block*-poly(ethylene glycol)-*block*- poly{(lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]}, is synthesized, in which the glucose molecules are attached to the PLGG blocks via an amide linkage. Its specific binding with Concanavalin A (Con A) is demonstrated.

Experimental Section

Materials. Dihydroxyl PEG with molecular weight of 2000 was obtained from Aldrich. Prior to use, it was dried by an azeotropic distillation in toluene. Palladium on activated charcoal (Pd/C, 10%) was received from Suzhou Xukou Chemical Corporation in China and used without further purification. *N*,*N*′-Carbonyldiimidazole (CDI) was purchased from Fluka. L-Lactide (LA) was prepared in our own laboratory and recrystallized from ethyl acetate three times before use. (3*s*)-3-Benzoxylcarbonylethylmorpholine-2,5-dione (BEMD) was syn-

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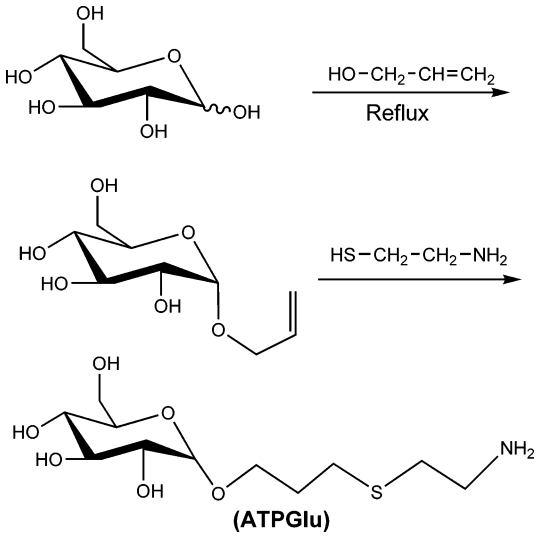
10.1021/bm0601225 CCC: $33.50
Published on Web 05/17/2006

Scheme 1. 3-(2-Aminoethylthio)propyl-α-D-glucopyranoside (ATPGlu)



thesized according to the literature.[36] Concanavalin A (Con A) and fluorescein isothiocyanate (FITC) were purchased from Sigma and used as received. Chloroform and dimethyl sulfoxide (DMSO) were refluxed over CaH_2 and distilled under argon. Membrane bags (molecular weight cutoff = 3000) used for dialysis were purchased from Shanghai Green Bird Technology Co., Ltd. Cation-exchange resin of Na^+ form (Brand 001 × 7(732), abbreviated as Resin 732 hereafter) was purchased from Sinopharm Shanghai Chemical Reagent Co., Ltd., and was converted into the acid and ammonium forms according to the literature.[37]

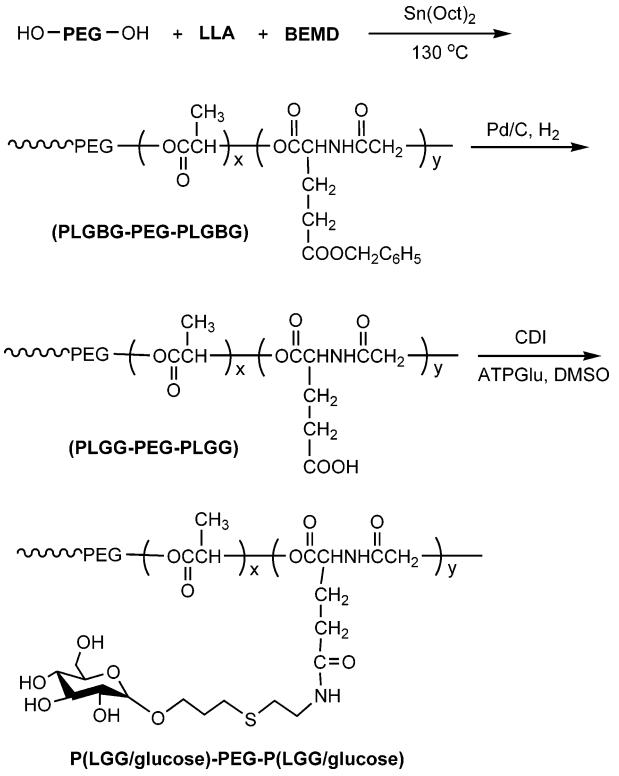
Measurements. FT-IR spectra were recorded on a Bio-Rad Win-IR instrument. Thin-layer chromatography (TLC) was performed on silica gel (60 F-254) of 0.2 mm in layer thickness. The column chromatography was carried out on silica gel. ^{1}H NMR spectra were measured by a Unity-400 NMR spectrometer at room temperature, with $CDCl_3$ and DMSO-d_6 as solvents and TMS as internal reference. Size-exclusion chromatography (SEC) measurements were conducted at 35 °C with a Waters 410 SEC instrument equipped with two Waters Styragel columns (HT6E, HT3) and a differential refractometer detector. $CHCl_3$ was used as eluant at a flow rate of 1.0 mL min^{-1}. CLSM pictures were taken on a Confocal Laser Scanning Microscope (FV 500, Olympus).

Synthesis of 3-(2-Aminoethylthio)propyl-α-D-glucopyranoside (ATPGlu).[37] ATPGlu was prepared according to the procedure in Scheme 1. To a suspension of D-glucose (5.0 g) in allyl alcohol (60 mL) was added the Resin 732 (in H^+ form) (4 g, 80−100 mesh), and the reaction mixture was refluxed at 110 °C. The reaction was monitored by TLC method (with mobile phase of ethyl acetate−2-propanol−water, 9:4:2; R_f = 0.43). After the D-glucose was converted into allyl-α-D-glucopyranoside, the resin was filtered out, and the filtrate was evaporated to syrup and used in the next step without further purification.

A solution of the above glycoside and cysteamine hydrochloride in deoxygenated water was stirred overnight at room temperature under argon atmosphere. The product was purified on a column of Resin 732 (NH_4^+ form) and washed with a linear gradient 0−1.0 M NH_4OH in water. Fractions containing the desired product (detected by TLC method, mobile phase: ethyl acetate−glacial acetic acid−water, 3:2:1, R_f = 0.35) were collected and evaporated. The product was finally lyophilized to afford a solid of 1.05 g (yield: 21%, based on D-glucose mass). ^{1}H NMR (D_2O): δ 4.82 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.06−3.15 (m, 8H, CHs in sugar residue and CH_2 of propyl spacer), 2.92 (t, 2H, CH_2), 2.65 (t, J = 6.6 Hz, 2H, CH_2), 2.56 (m, J = 7.2 Hz, 2H, CH_2), 1.86 (m, 2H, CH_2). Anal. Calcd for $C_{11}H_{23}NO_6S$ (297.37): C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.30; H, 7.89; N, 4.70; S, 10.82.

Synthesis of PLGG-PEG-PLGG.[38] The copolymer PLGG-PEG-PLGG was prepared by ring-opening polymerization (ROP) of LA and BEMD in the presence of dihydroxyl PEG as macroinitiator and by subsequent catalytic hydrogenation, as shown in Scheme 2. Briefly, the PEG (0.528 g) in a polymerization vessel was dried by an azeotropic distillation in toluene for 30 min. The solution was allowed to cool to ambient temperature, and the toluene was evaporated completely under reduced pressure. Then, under the protection of argon, prescribed amounts of LA (2.236 g, 15.53 mmol), BEMD (0.423 g, 1.52 mmol), and $Sn(Oct)_2$ (0.65 mL, 2.65 × 10^{-2} mol L^{-1}) were added. The vessel was degassed by several vacuum-argon purge cycles, sealed, and placed in an oil bath at 130 °C for 48 h. The polymerization was terminated by cooling to room temperature. The copolymers were dissolved in a small amount of chloroform, precipitated into excess cold methanol, isolated by filtration, and dried in a vacuum at room temperature. The removal of the protecting benzyl groups was realized easily according to reference.[38] The deprotected copolymer was dried in a vacuum at room temperature.

Scheme 2. Synthesis of Glucose-Grafted PLGG-PEG-PLGG Triblock Copolymers



Preparation of Glucose-Grafted PLGG-PEG-PLGG. Under dried argon atmosphere, 0.1 g (28.6 μmol COOH) of copolymer PLGG-PEG-PLGG was dissolved in 2 mL of DMSO, and the solution was added to another flask which contained a solution of 6.5 mg (37.1 μmol) of CDI in 1 mL of DMSO. The mixture was stirred for 24 h at 60 °C. A quantity (16.9 mg, 57.1 μmol) of ATPGlu dissolved in 2 mL of DMSO was added to the above copolymer solution. The reaction mixture was stirred at 60 °C for another 24 h. After cooling to room temperature, the reaction mixture was concentrated and precipitated into excess of CH_3OH. The polymer P(LGG/glucose)-PEG-P(LGG/glucose) was collected by centrifugation and washed with ethanol three times and finally dried under high vacuum at room temperature for 24 h.

Cytotoxicity of P(LGG/glucose)-PEG-P(LGG/glucose). Human umbilical vein endothelial cells (ECV-304) were used to investigate cytotoxicity of P(LGG/glucose)-PEG-P(LGG/glucose). ECV-304 cells were first maintained in MEM medium containing 50 mg L^{-1} vitamin C and 10% calf serum, and the culture medium was replaced once every day. The P(LGG/glucose)-PEG-P(LGG/glucose) was dissolved in $CHCl_3$ (1%), and the solution was cast onto glass slides. The solvent was removed under a vacuum for 48 h to form copolymer films. The specimens were placed in the culture wells, and then, the MEM medium

was added into each well. The cells were seeded in each well with a cell density of 1×10^5 cells/well and were incubated at 37 °C in 5% CO_2. The growth medium was replaced with fresh medium once every 24 h. The same experiments were performed on PLA films and glass slides for comparison. The status of adhesion and growth of the cells was observed at 4 and 24 h, respectively, by an inverted fluorescence microscope (TE2000-U, Nikon).

Specific Binding. Concanavalin A (Con A) labeled with FITC was used as a glucose-binding protein to study specific binding ability of the glucose-grafted copolymer. The solution of Con A (2 mg, 0.019 μmol) and FITC (40 μg) in 10 mL of 10% PBS was stirred for 2 h under dark condition at room temperature. The unreacted FITC was removed by dialysis in a pre-swollen membrane bag (molecular weight cutoff = 3000). The copolymer PLGG-PEG-PLGG and P(LGG/glucose)-PEG-P(LGG/glucose) were cast from their chloroform solutions into films. After being treated in water of 80 °C for 10 min, the films were cooled to room temperature and placed in 2 mL of Con A solution in PBS (0.2 mg/mL). Two hours later, the film was fully washed with distilled water three times and examined by a confocal laser scanning microscope (CLSM).

Results and Discussion

Synthesis of 3-(2-aminoethylthio)propyl-α-D-glucopyranoside (ATPGlu). To graft D-glucose onto PLGG-PEG-PLGG which contained reactive carboxyl groups in the PLGG blocks, it should be converted into OH- or NH_2-terminated molecules. In the present study, allyl glycoside is reacted with cysteamine to get ATPGlu with the terminal amino group. Another advantage of this synthetic route is to provide a longer spacer between the glucose residue to the main chain, which is necessary to exclude possible interference from the main chain to the glucose function. The synthetic reactions are shown in Scheme 1.[39−41] D-Glucose was refluxed with a large amount of allyl alcohol under the catalysis of Resin 732 (H^+) to obtain crude allyl α-glycoside. After purification by column chromatography using 0.1 M acetic acid as solvent and eluant, the allyl α-glycoside was reacted with cysteamine in deoxygenated water to get ATPGlu. The total yield was 25% based on the D-glucose mass.

Synthesis of PLGG-PEG-PLGG. The PEG containing two hydroxyl endgroups is often used as a starting material for synthesis of block copolymers. It was convenient for us to synthesize copolymer PLGBG-PEG-PLGBG by the polymerization of LLA and BEMD with stannous octoate ($SnOct_2$) as catalyst in the presence of PEG as macroinitiator. The copolymer was easily debenzylated by catalytic hydrogenolysis under H_2 condition over Pd/C (10%) as catalyst in a mixture solvent of THF/methanol at 50 °C for 48 h. As shown in Figure la, the resonances of benzyl group at 7.34 ppm (C_6H_5) in the copolymer PLGBG-PEG-PLGBG completely disappeared after the hydrogenolysis reaction, while other resonances of the copolymer did not change. The disappearance of the δ_{CH} vibration of the benzyl group at 756 and 701 cm^{-1} in Figure 2 was further evidence for the successful debenzylation. The molecular chain structure of the copolymers obtained was not changed. The molecular weight of PLGG-PEG-PLGG was estimated by SEC (Table 1) to be 8.57×10^3, which was larger than that of its parent copolymer PLGBG-PEG-PLGBG, probably due to the change in COOH functionality. Approximately, every copolymer chain contained four carboxyl groups according to the ^1H NMR spectra.

Synthesis of Glucose-Grafted PLGG-PEG-PLGG. The pendant carboxyl groups on the copolymer PLGG-PEG-PLGG provided the possibility of reacting with the terminal amino group of ATPGlu. As shown in Scheme 2, the condensation was performed with the help of CDI, which activated the carboxyl group to form carboxylic acid imidazolide that combined with the amino group to form an amide linkage. This method is one of the simplest and most widely used pathways for a variety of coupling reactions. In addition, the byproducts of the reaction were only CO_2 and imidazole, which are nontoxic.[42] A conversion of the carboxyl groups as high as 94.6% was achieved according to the ^1H NMR spectra. As shown in Figure 1, the characteristic peaks of the PLGG-PEG-PLGG (a) and ATPGlu (b) could be observed in ^1H NMR spectra of P(LGG/glucose)-PEG-P(LGG/glucose) (c), which indicated the successful binding of ATPGlu to the pendant carboxyl groups of PLGG-PEG-PLGG. Furthermore, molecular weight of the glucose-grafted copolymer P(LGG/glucose)-PEG-P(LGG/glucose) (Table 1) became higher than that of PLGG-PEG-PLGG, because the mass of ATPGlu was added to the copolymer, and part of the COOH groups were replaced by the amide bonds.

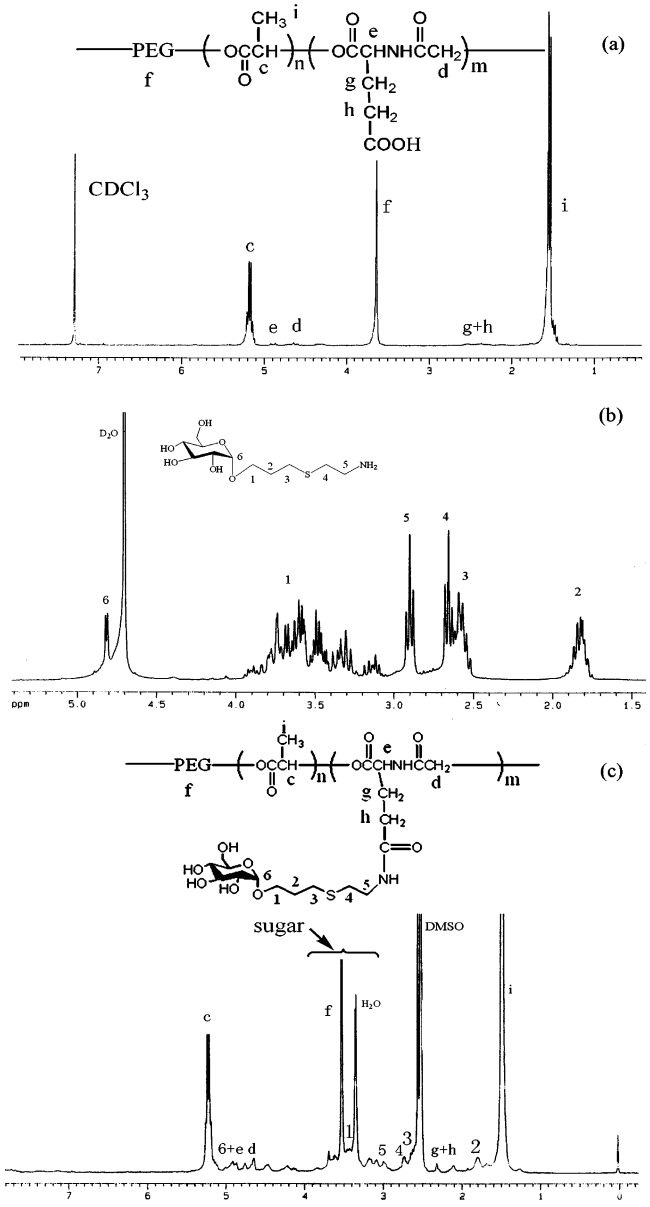


Figure 1. ^1H NMR spectra of PLGG-PEG-PLGG (a), 3-(2-aminoethylthio)propyl-α-D-glucopyranoside (b), and P(LGG/glucose)-PEG-P(LGG/glucose) (c).

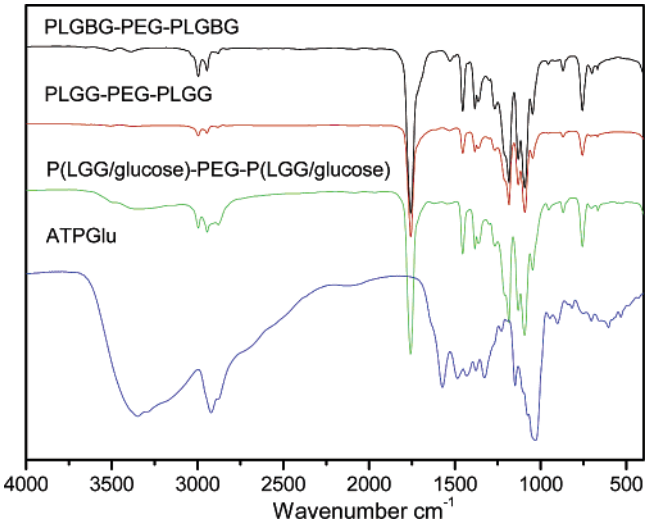


Figure 2. FT-IR spectra of PLGBG-PEG-PLGBG, PLGG-PEG-PLGG, P(LGG/glucose)-PEG-P(LGG/glucose), and ATPGlu.

Table 1. Compositions and Molecular Weights of the Investigated Copolymers

copolymer	mol. content of BEMD unit[a] (%)	$M_n(10^3)$[b]	M_w/M_n[b]
PLGBG-PEG-PLGBG	4.0	7.55	1.51
PLGG-PEG-PLGG	4.0	8.57	1.46
P(LGG/glucose)-PEG-P(LGG/glucose)	4.0	9.48	1.64

[a] Determined by ^{1}H NMR ($CDCl_3$). [b] Determined by SEC ($CHCl_3$ as eluant).

It should be pointed out that, in the above allylation−amination−amidation approach, it is not necessary to protect the hydroxyl groups of the glucose. These hydroxyl groups do not interfere with the reactions. Therefore, this approach may be used in similar synthesis of carbohydrate derivatives.

Biocompatibility of the Glucose-Grafted Copolymer. The in vitro cytotoxicity of P(LGG/glucose)-PEG-P(LGG/glucose) was evaluated qualitatively. First, it was dissolved in $CHCl_3$ and cast into films on glass slides. Second, the ECV-304 cells were seeded onto these polymer films and cultured in MEM medium in cultural wells. Third, the specimens were observed at the designated time intervals, and the adhesion and growth behaviors of the cells were taken with a DXM1200F digital camera (Nikon). Figure 3 shows the micrographs of the ECV-304 cells incubated for 4 and 24 h. After 4 h, some cell spreading was seen on the polymer film. After 24 h, almost all cells got spread and began to proliferate, and their density increased gradually and finally occupied the whole polymer surface. The same experiments were carried out for PLA films and glass slides for comparison. As shown in Figure 3, density of cells adhering to the P(LGG/glucose)-PEG-P(LGG/glucose) film was greater than that on PLA film, and they grew faster in the same time. This improvement was attributed to the improved hydrophilicity of the P(LGG/glucose)-PEG-P(LGG/glucose) film surface because of the presence of the glucose residues and the PEG segments. Therefore, the MEM culture provided an encouraging result regarding the biocompatibility of this glucose-grafted copolymer and suggested its further application in biomedical fields such as tissue engineering.

Specificity in Protein Binding. To assess the activity of the glucose-grafted triblock copolymer, Con A was chosen as a target protein because it is a well-known glucose-binding protein and exists as a homotetramer at neutral pH. It has four binding sites and can interact with four glucose units simultaneously.[43−45]

The PLGG-PEG-PLGG and P(LGG/glucose)-PEG-P(LGG/glucose) were cast into colorless films. And the films were further treated in hot water at 80 °C for 10 min. Because 80 °C is higher than the glass transition temperature of the copolymer, the PEG segments and glucose residues were supposed to migrate to the film surfaces to endue the films with improved hydrophilicity. Additionally, the PBS solution of Con A is maintained on the surface of the films at 25 °C. After 2 h, the films were washed with distilled water three times to get rid of any unbound Con A on the surface. Because Con A was labeled by fluorescent dye FITC, Con A could be visualized under CLSM. As shown in Figure 4a, there were no fluorescent signals

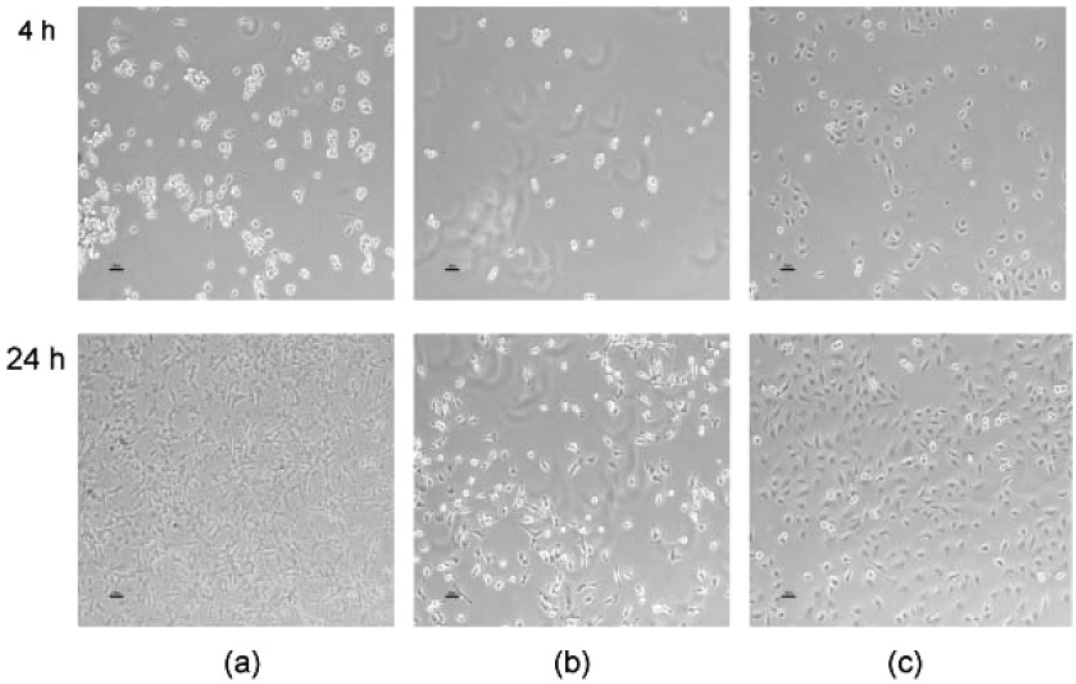


Figure 3. Micrographs of ECV-304 seeded on (a) P(LGG/glucose)-PEG-P(LGG/glucose) film, (b) PLA film, and (c) a glass slide after incubation for 4 and 24 h.

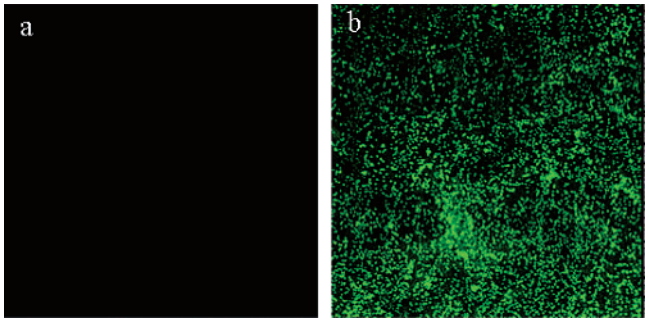


Figure 4. CLSM micrographs of PLGG-PEG-PLGG film (a) and P(LGG/glucose)-PEG-P(LGG/glucose) film (b) after treatment with FITC-labeled Con A.

on the PLGG-PEG-PLGG film, while there were many fluorescent spots on the P(LGG/glucose)-PEG-P(LGG/glucose) film in Figure 4b. Obviously, this difference was not due to the presence of PEG segments in the copolymers, because both PLGG-PEG-PLGG and P(LGG/glucose)-PEG-P(LGG/glucose) contained the same amount of PEG. The glucose residues were supposed to be responsible for the enhanced fluorescence of P(LGG/glucose)-PEG-P(LGG/glucose), because they reacted with FITC-labeled Con A molecules to fix them on the film surface. Therefore, the CLSM observation provided powerful evidence for the specific binding of the glucose-grafted copolymer with Con A.

Conclusion

A novel glucose-grafted copolymer P(LGG/glucose)-PEG-P(LGG/glucose) was prepared in three steps: (1) An amphiphilic block copolymer PLGBG-PEG-PLGBG with pendant benzyl ester groups was synthesized by ROP of LLA and BEMD in the presence of dihydroxyl PEG as macroiniator. (2) The protecting benzyl groups on the copolymer were removed by catalytic hydrogenation to get triblock copolymer PLGG-PEG-PLGG with pendant carboxyl groups. (3) 3-(2-Aminoethylthio)-propyl-α-D-glucopyranoside was attached covalently to the pendant carboxyl groups of PLGG-PEG-PLGG with the help of CDI. The resultant glucose-grafted copolymer film showed lower cytotoxicity against ECV-304 cells compared to PLA film and improved binding ability with Con A molecules than PLGG-PEG-PLGG. Its selective recognition and specific interaction with protein molecules are expected to find applications in biomedical fields, especially in drug delivery and tissue engineering.

Acknowledgment. The project is financially supported by the National Natural Science Foundation of China (project nos. 20274048 and 50373043), by the National Fund for the Distinguished Young Scholars (no. 50425309), and by Chinese Academy of Sciences (project no. KJCX2-SW-H07).

References and Notes

(1) Chabot, F.; Vert, M.; Chapelle, S.; Granger, P. *Polymer* **1983**, *24*, 53.
(2) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem. Rev.* **1999**, *99*, 3181.
(3) Chiellini, E.; Solaro, R. *Adv. Mater.* **1996**, *8*, 305.
(4) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature (London)* **1997**, *388*, 860.
(5) Kowalski, A.; Duda, A.; Pencaek, S. *Macromolecules* **2000**, *33*, 689.
(6) Kricheldorf, H. R.; Berl, M.; Scharnagl, N. *Macromolecules* **1988**, *21*, 286.
(7) Cook, A. D.; Hrkach, J. S.; Gao, N. N.; Johnson, I. M.; Pajvani, U. B.; Canizzaro, S. M.; Langer, R. *J. Biomed. Mater. Res.* **1997**, *35*, 513.
(8) Yamaoka, T.; Hotta, Y.; Hotta, Y.; Kobayashi, K.; Kimura, Y. *Int. J. Biol. Macromol.* **1999**, *25*, 265.
(9) Shin, H.; Jo, S.; Mikos, A. G. *J. Biomed. Mater. Res.* **2002**, *61*, 169.
(10) Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. *J. Am. Chem. Soc.* **1993**, *115*, 11010.
(11) Langer, R. *Macromolecules* **1995**, *28*, 425−432.
(12) Kricheldorf, J. R.; Fechner, B. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 1047.
(13) Lou, X.; Detrembleur, C.; Jérôme, R. *Macromol. Rapid Commun.* **2003**, *24*, 161.
(14) Guan, H. L.; Deng, C.; Xu, X. Y.; Liang, Q. Z.; Chen, X. S.; Jing, X. B. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 1144.
(15) Finne, A.; Albertsson, A. C. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 444.
(16) Saulnier, B.; Ponsart, S.; Coudane, J.; Garreau, H.; Vert, M. *Macromol. Biosci.* **2004**, *4*, 232.
(17) Riva, R.; Schmeits, S.; Stoffelbach, F.; Jérôme, C.; Jérôme, R.; Lecomte, P. *J. Chem. Soc., Chem. Commun.* **2005**, 5334.
(18) Liu, L.; Li, X. C.; Yuan, Z.; An, Y. L.; He, B. L. *J. Appl. Polym. Sci.* **2001**, *80*, 1976.
(19) Adams, M. L.; Andes, D. R.; Kwon, G. S. *Biomacromolecules* **2003**, *4*, 750.
(20) Richter, A. W.; Akerblom, E. *Int. Arch. Allergy Appl. Immunol.* **1983**, *70*, 124.
(21) Tang, Y.; Liu, S. Y.; Armes, S. P.; Billingham, N. C. *Biomacromolecules* **2003**, *4*, 1636.
(22) Schleepper-Schäfer, J.; Hülsmann, D.; Djovkar, A.; Meyer, H. E.; Herbertz, L.; Kolb, H.; Kolb-Bchofen, V. *Exp. Cell Res.* **1986**, *165*, 494.
(23) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.
(24) Varki, A. *Glycobiology* **1993**, *3*, 97.
(25) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71.
(26) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692.
(27) Lee, Y.; Lee, R. *Acc. Chem. Res.* **1995**, *28*, 321.
(28) Bovin, N. V.; Gabius, H. J. *Chem. Soc. Rev.* **1995**, *24*, 413.
(29) (a) Roy, R. In *Carbohydrate Chemistry*; Boons, G. J., Ed.; Blackie Academic & Professional: London, 1998; p 243. (b) Horejsl, V.; Kocourek, J. *Biochem. Biophys. Acta* **1973**, *297*, 346.
(30) (a) Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 4179. (b) Bovin, N. V.; Yu, E.; Zemlyanukhina, K. T. V.; Byramova, N. E.; Galanina, O. E.; Zemylakov, A. E.; Ivanov, A. E.; Zubov, V. P.; Machalova, L. V. *Glycoconjugate J.* **1993**, *10*, 142. (c) Baek, M. G.; Roy, R. *Macromol. Biosci.* **2001**, *1*, 305.
(31) Yasugi, K.; Nkamura, T.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Macromolecules* **1999**, *32*, 8024.
(32) Nagasaki, Y.; Okada, T.; Scholz, C.; Iijima, M.; Kato, M.; Kataoka, K. *Macromolecules* **1998**, *31*, 1473.
(33) Jule, E.; Nagasaki, Y.; Kataoka, K. *Bioconjugate Chem.* **2003**, *14*, 177.
(34) Cammas, S.; Kataoka, K. *Macromol. Chem. Phys.* **1995**, *196*, 1899.
(35) (a) DeFrees, S. A.; Gaeta, F. C. A.; Lin, U. C.; Ichikawa, Y.; Wong, C. H. *J. Am. Chem. Soc.* **1993**, *115*, 7549. (b) Matrosovich, M. N.; Mochalova, L. S.; Marinina, V. P.; Byramova, N. E.; Bovin, N. V. *FEBS Lett.* **1990**, *272*, 209. (c) Glick, G. D.; Toogood, P. L.; Wiley, D. C.; Skehel, J. J.; Knowles, J. R. *J. Biol. Chem.* **1991**, *266*, 23660.
(36) Deng, X.; Yao, J.; Yuan, M.; Li, X.; Xiong, C. *Macromol. Chem. Phys.* **2000**, *201*, 2371.
(37) Lee, R. T.; Lee, Y. C. *Carbohydr. Res.* **1974**, *37*, 193.
(38) Guan, H.; Xie, Z.; Zhang, P.; Deng, C.; Chen, X.; Jing, X. *Biomacromolecules* **2005**, *6*, 1955.
(39) Peter, M. G.; Boldt, P. C.; Peterson, S. *Liebigs Ann. Chem.* **1992**, 1275.
(40) Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *Tetrahedron* **1992**, *48*, 4713.
(41) Konradsson, P.; Roberts, C.; Fraser-Reid, B. *Recl. Trav. Chim. Pays Bas* **1991**, *110*, 23.
(42) (a) Liebert, T. F.; Heinze, T. *Biomacromolecules* **2005**, *6*, 333. (b) Staab, H. A. *Angew Chem.* **1962**, *12*, 407.
(43) *Concanavalin A as a tool*; Bittiger, H., Schnebli, H. P., Eds.; John Wiley & Sons, Ltd.: London, 1976.
(44) Derevenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb, A. J.; Dodson, E. J.; Papix, M. Z.; Wan, T.; Campbell, J. *EMBO J.* **1989**, *8*, 2189.
(45) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053.

BM0601225