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Hyperbranched Double Hydrophilic Block Copolymer Micelles of Poly(ethylene oxide) and Polyglycerol for pH-Responsive Drug Delivery

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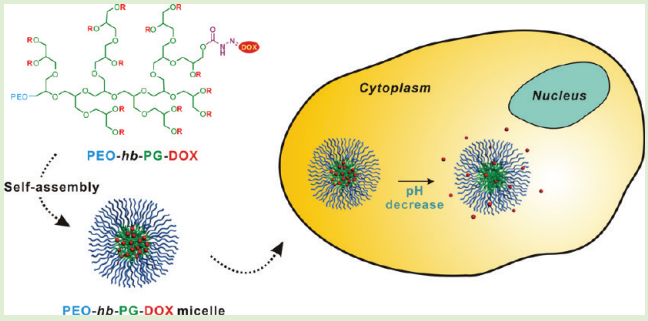
Supporting Information

ABSTRACT: We report the synthesis of a well-defined hyperbranched double hydrophilic block copolymer of poly(ethylene oxide)-*hyperbranched*-polyglycerol (PEO-*hb*-PG) to develop an efficient drug delivery system. In specific, we demonstrate the hyperbranched PEO-*hb*-PG can form a self-assembled micellar structure on conjugation with the hydrophobic anticancer agent doxorubicin, which is linked to the polymer by pH-sensitive hydrazone bonds, resulting in a pH-responsive controlled release of doxorubicin. Dynamic light scattering, atomic force microscopy, and transmission electron microscopy demonstrated successful formation of the spherical core−shell type micelles with an average size of about 200 nm. Moreover, the pH-responsive release of doxorubicin and in vitro cytotoxicity studies revealed the controlled stimuli-responsive drug delivery system desirable for enhanced efficiency. Benefiting from many desirable features of hyperbranched double hydrophilic block copolymers such as enhanced biocompatibility, increased water solubility, and drug loading efficiency as well as improved clearance of the polymer after drug release, we believe that double hydrophilic block copolymer will provide a versatile platform to develop excellent drug delivery systems for effective treatment of cancer.



INTRODUCTION

Various drug delivery approaches have been developed to achieve site-specific and time-controlled delivery of therapeutics to improve therapeutic efficacy while minimizing undesired side effects.[1] Self-assembled nanostructures have long been studied as promising vehicles for the delivery of active therapeutics. Recently, self-assembled polymeric micelles have been extensively utilized in drug delivery systems because of their unique features, such as high loading capacity and enhanced solubility of drugs, reduced systemic adverse effects, preferential accumulation at the tumor site owing to enhanced permeability and retention (EPR) effect, and perhaps, most importantly, high tunability of chemical and physical characteristics with respect to the field of applications.[2−5] Despite these advantages of polymeric micelles, the sophisticated delivery of active therapeutics from the carriers remains a challenge in the development of advanced drug delivery systems.

As alternatives to traditional micelle systems, smart nanocarriers are actively pursued that can stably encapsulate therapeutics and release them at a desired site in response to external stimuli such as pH, temperature, redox, and light.[6−9] For example, the Kataoka group has extensively studied the development of intracellular pH-triggered polymeric micelles conjugated with various chemotherapeutic agents in a series of papers.[10−13] One approach for the development of pH-sensitive polymer micelles is to covalently conjugate drugs to the polymer via acid degradable linkages such as hydrazone, ester, and carbamate.[14−16] When the pH changes, the linkages degrade with concomitantly releasing the drug from the micelle at a specific location. Compared to the traditional approaches in which the drug is physically entrapped in the hydrophobic core of micelles by hydrophobic interactions, the aforementioned approach enables control and modification of the drug release rate from these micelles, as proved in the case of smart nanocarrier systems.[17]

To date, various pH-responsive polymeric micelles have been successfully reported; however, only a few examples have advanced to the clinical settings, mostly due to their intrinsic toxicity. To resolve the issues associated with toxicity of the carrier, many biocompatible polymers such as poly(ethylene oxide), polyglycerols (or polyglycidols), and sugar derivatives together with biodegradable polymers like polyester, poly-

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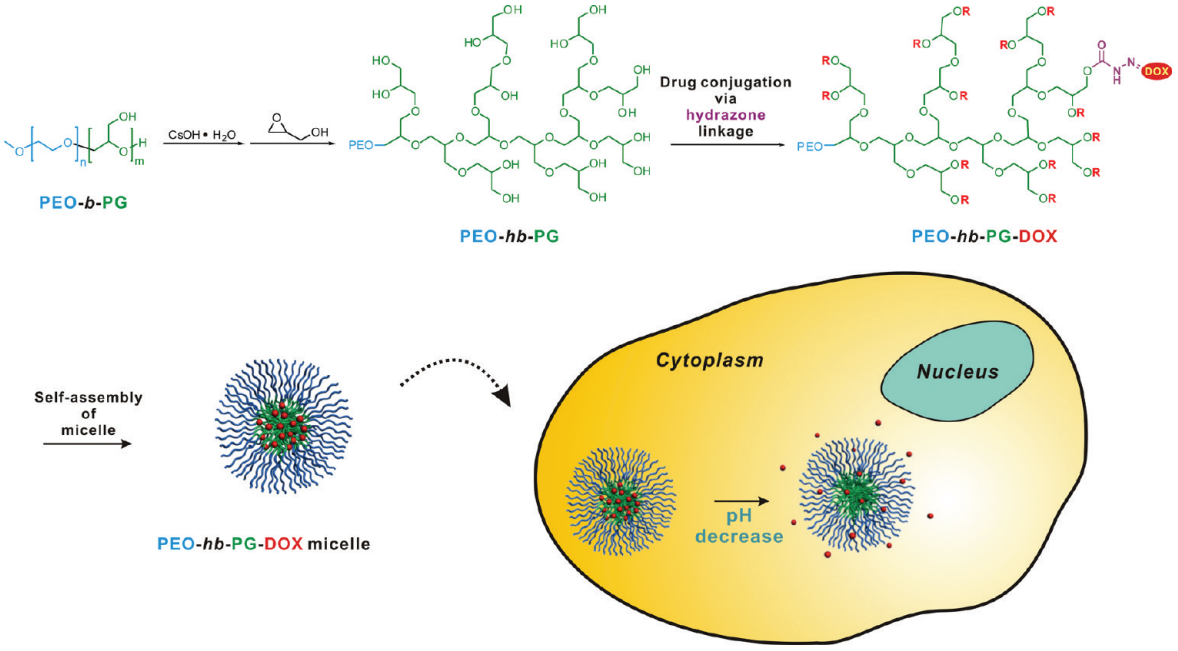
Scheme 1. Illustration of Hyperbranched Double Hydrophilic Block Copolymer Conjugated with Doxorubicin (DOX) and Its Self-Assembled Micelle for the pH-Responsive Intracellular Release of Active Therapeutics



carbonate, and polypeptides are often employed as the polymeric segments to form micelles in aqueous solutions.

Polyglycerols are a class of hyperbranched polyethers with excellent biocompatibility. Although initial synthetic approaches for constructing polyglycerols were rather challenging, the recent advancement by Sunder and co-workers allows the synthesis of well-defined and complex architectures of polyglycerols with relatively low polydispersity (M_w/M_n = 1.2−1.9).[18,19] They also exhibit flexibility in physicochemical properties by the convenient end-group functionalizations. Recently, polyglycerols with varying architectures have been designed and synthesized for biomedical applications.[20−25] Brooks and co-workers have extensively investigated the suitability of hydrophobically functionalized hyperbranched polyglycerols for use as synthetic albumin substitutes and as general drug delivery vehicles.[23−25]

In this article, we designed the hyperbranched double hydrophilic block copolymer of poly(ethylene oxide)-*hyperbranched*-polyglycerols (PEO-*hb*-PG) with an aim to enhance biocompatibility, to increase water solubility, and to improve the clearance of the polymer after drug release.[25] Moreover, the hyperbranched architecture of polyglycerol can potentially increase the loading capacity of active therapeutics that are linked by the pH-responsive hydrazone moiety for an effective drug delivery system.[26] We found that hyperbranched PEO-*hb*-PG can form a self-assembled micellar structure upon conjugation with hydrophobic therapeutics, which in turn exhibits a pH-responsive controlled release of doxorubicin (Scheme 1).

EXPERIMENTAL SECTION

Materials. Cesium hydroxide monohydrate, poly(ethylene oxide) monomethyl ether (nominal number average molecular weight (M_n) of 2000) (PEO), doxorubicin hydrochloride, *N*,*N*′-dimethylformamide (DMF), triethylamine, dimethylsulfoxide, and hydrazine monohydrate were purchased from Sigma-Aldrich. *p*-Nitrophenyl chloroformate (*p*-NPC) was purchased from TCI (Japan). 1,4-Dioxane was dried over sodium and distilled prior to use. For polymerization, diglyme (99%, Acros) and methoxy ethanol (99.5%, Acros) were purified by distillation from CaH_2 directly prior to use. The cation-exchange resin, Dowex 50W × 2 (50−100 mesh) was purchased from Wako Pure Chemical (Japan) and used after washing with methanol. Other reagents and solvents were used as received.

Measurements. ^{1}H NMR spectroscopy (VNMRS 600 spectrometer 600 MHz, Varian, U.S.A.) was used with DMSO-d_6 as the solvent. M_n and M_w were measured by a gel permeation chromatography (GPC) on Tosoh HLC-8120 GPC equipped with a consecutive polystyrene gel column (TSK-GEL GMHHR-M and GMHHR-N) at 40 °C by eluting with *N*-methyl-2-pyrrolidone (NMP) containing 0.01 M lithium bromide at a flow rate of 1.0 mL/min, which was calibrated by standard polystyrene samples. Transmission electron microscopy (TEM, JEM-2100, JEOL, Japan) and atomic force microscopy (AFM, Dimension 3100, Veeco, U.S.A.) were performed to investigate the morphology of the PEO-*hb*-PG micelles. Size distribution analysis was studied using dynamic light scattering (DLS, Nano ZS, Malvern, U.K., and BI-APD, Brookhaven Instrument, New York, U.S.A.). Two instruments were used to crosscheck the reliability of the obtained data. The amount of the released DOX was measured by high performance liquid chromatography (HPLC, 1200 series, Agilent, U.S.A.) with a mobile phase of a mixture of CH_3CN and H_2O (4:6, v/v) at a rate of 0.80 mL/min and 20 °C. The detection of DOX was performed using a UV detector at a wavelength of 480 nm.

Synthesis of Ethoxyethyl Glycidol Ether (EEGE). This compound was prepared by modifying a previously reported method.[27] Briefly, 85.00 g (1.147 mol) glycidol and 225.9 g (3.133 mol) ethyl vinyl ether were added to a 500 mL two-neck flask with a magnetic stirring bar. This mixture was cooled to −30 °C, and 1.915 g (11.12 mmol, 1.0 mol % to glycidol) *p*-toluene sulfonic acid monohydrate (TsOH) was slowly added. After the addition, the mixture was kept stirring at 25 °C for another 3 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution, and the organic layer was separated and dried with $MgSO_4$. After filtration, the residual ethyl vinyl ether was removed under reduced pressure, and the remainder was distilled in vacuo to yield EEGE as a colorless liquid product. Yield: 74%, b.p. 50 °C/0.6 Torr. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 1.10 (t, 3H, $-CH_3$), 1.19 (q, 2H, $-OCH_2CH_3$), 2.53−2.73 (m, 2H, CH_2 of the epoxy ring), 3.07−3.09 (m, 1H, CH of the epoxy ring), 3.25−3.77 (m, 4H, $-OCH_2CH_3$ and $-OCH_2-$), 4.69

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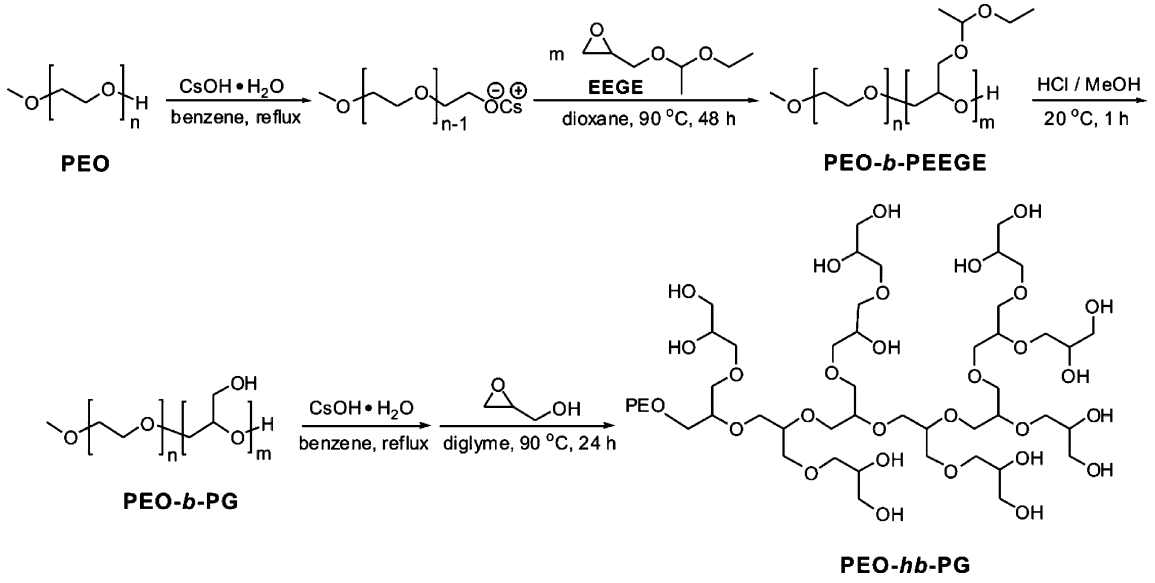
Scheme 2. Synthetic Approach for Preparation of PEO-*hb*-PG Copolymer



(m, -OCH(CH_3)O-) ppm. ^{13}C NMR (101 MHz, DMSO-d_6, ppm): δ 15.2, 19.8, 39.5, 43.6, 50.4, 60.3, 66.0 ppm.

Synthesis of PEO-*b*-PG. The linear block copolymer was prepared by the modified method in literature.[28] Briefly, 1.001 g (0.5826 mmol) of PEO (monomethyl ether, M_n = 1720, determined by GPC) and 0.072 g (0.429 mmol) of cesium hydroxide monohydrate were placed in 4 mL of benzene under nitrogen. The heterogeneous solution was stirred at 60 °C for 30 min, and the solvents were removed by a rotary evaporator. The opaque viscous liquid was heated at 90 °C, and dried under reduced pressure for 1 h. After nitrogen was introduced into this flask, 1 mL of dry 1,4-dioxane was added. Then, 0.980 g (6.70 mmol) of EEGE was added dropwise using a syringe in one portion, and the temperature was raised to 90 °C for 2 days. The reaction mixture was cooled to 50 °C, and the polymerization was terminated by the addition of 10 mL of methanol containing an acidic ion-exchange resin (ca. 9 g). This mixture was filtered and poured into cold diethyl ether to yield the acetal-protected block copolymer. HCl solution (1.0 M, ca. 100 mL) in methanol was added dropwise into 4 mL of polymer solution in ethanol (ca. 20 wt %), and the mixture was stirred for 1 h. Then, potassium carbonate was carefully added to neutralize the solution (pH was checked with pH test paper). Filtration and precipitation in diethyl ether yielded the pure PEO-*b*-PG block copolymer which was dried in vacuo at 80 °C for 2 days with a yield of 0.888 g (59%). The block efficiency (BE) of PEO-*b*-PG is calculated using the following equation: BE = reacted PEO/used PEO = (0.888 − 0.497 g)/1.001 g = 0.39. In addition, the M_n of the block copolymer PEO-*b*-PG can be calculated to be 3900 g/mol, obtained from the following equation: M_n (PEO) + M_n (PG) = 1720 + EEGE/reacted PEO (in mol) × M_w (glycidol) = 1720 + 6.70/(0.391 g/1720 g/mol × 1000) × 74.08.

Synthesis of PEO-*hb*-PG. The hypergrafting of the linear block copolymer PEO-*b*-PG was performed by using the Frey's method.[28] The linear macroinitiator PEO-*b*-PG (0.854 g, 6.45 mmol as OH function) was placed in a two-neck flask and dissolved in benzene (3.5 mL, ca. 20 wt %). Cesium hydroxide monohydrate (0.270 g, 1.61 mmol) was added to achieve deprotonation of 25% of the hydroxyl groups along the backbone. The heterogeneous solution was stirred at 60 °C for 30 min, and the solvents were removed by a rotary evaporator. The opaque viscous liquid was heated at 90 °C and dried under reduced pressure for 1 h. After nitrogen was introduced into this flask, dry 3.5 mL of diglyme was added (ca. 20 wt %). The flask was placed in an ultrasonic bath for 30 min, and the mixture was heated to 90 °C. Then, glycidol (2.062 g, 27.83 mmol) in 8.5 mL of diglyme was slowly added with a syringe over a period of approximately 24 h. The reaction was terminated by the addition of excess methanol containing an acidic cation exchange resin. This mixture was filtered and poured into cold diethyl ether; the precipitate was dried at 40 °C for 2 days to obtain the hypergrafted PEO-*hb*-PG copolymer with a yield of 1.88 g (65%). From the yield we calculated the weight of PG in PEO-*hb*-PG to be 1.026 g (13.85 mmol) by using the following equation: mass of PG in PEO-*hb*-PG = (mass of PEO-*hb*-PG) − (mass of used PEO-*b*-PG) = 1.88 − 0.854 g = 1.026 g. The numbers of OH group in PEO-*b*-PG was determined to be 6.788 mmol, which was calculated from the equation: 0.854 g (weight of PEO-*b*-PG)/3900 (M_n of PEO-*b*-PG) × 100 × (29.5 + 1) (repeating unit of PG + terminal OH). Therefore, the M_n of the final polymer can be estimated to be 8580, from 3900 (M_n of PEO-*b*-PG) + 13.85 mmol/0.2190 mmol × 74.08 (number of PG unit per prepolymer × M_w of (glycidol)), which shows good agreement with the GPC data of 9300. Finally, we determined the number of OH group per polymer chain to be 29.5 + 63.2 + 1 = 93.

Conjugation of Doxorubicin to PEO-*hb*-PG. PEO-*hb*-PG copolymer (100 mg, 1.09 mmol based on the number of OH groups) was dissolved in 5 mL of DMF and triethylamine (0.160 mL, 1.15 mmol) was added in the solution. The copolymer solution was dropped into *p*-NPC (11.5 mmol, 2.32 g, 10 equiv) solution in 20 mL of DMF for 1 h, and reacted for additional 24 h with stirring. After methanol addition to remove unreacted *p*-NPC, the solvent was removed by a rotary evaporator. The product was then precipitated in ether and dried. The *p*-NPC conjugated PEO-*hb*-PG (100 mg, 1.09 mmol) dissolved in 10 mL of DMF was added slowly into hydrazine monohydrate solution (11.5 mmol, 3.6 mL, 10 equiv) in 5 mL of DMF and stirred overnight. After DMF was removed in vacuo, the product was dissolved in deionized water, followed by dialyzing against water. After removal of deionized water, the residue and 10 mg (0.017 mmol) doxorubicin (DOX) were dissolved in 10 mL of DMF. The mixture was kept stirring for 48 h at room temperature.[29]

Preparation of PEO-*hb*-PG-DOX Micelles. DOX-loaded PEO-*hb*-PG micelles (PEO-*hb*-PG-DOX) were prepared by dialysis against deionized water pH-adjusted to 9 for 12 h, to remove any residual solvent and unreacted DOX.

Doxorubicin Release. A 4 mL solution of PEO-*hb*-PG-DOX micelles was placed inside a dialysis membrane of molecular weight cutoff (3500−5000 Da) and dialyzed against either pH 5.0 or pH 7.4 phosphate buffer solution (solution mixture of appropriate volume of 10 mM KH_2PO_4 and K_2HPO_4). The micelles solution inside the membrane was collected at predetermined time points and the amount of the released doxorubicin was measured using HPLC with a standard calibration curve constructed using a UV/vis detector at a wavelength of 480 nm.

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In Vitro Cell Cytotoxicity. Cell viability was assessed by the standard MTT assay with slight modifications.[30] Briefly, cells were grown in 96 well plates at a density of 2×10^4 cells per well. After the treatment of the micelles as indicated concentrations, thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich) was added to each well of cells (final conc. 0.5 mg/mL) and incubated for 1.5 h at 37 °C in a humidified atmosphere of 95% air/5% CO_2. A solution of 0.08 N HCl in 2-isopropanol was added to solubilize the blue MTT-formazan product and the sample was incubated for further 30 min at room temperature. Absorbance of the solution was read at a test wavelength of 550 nm. Half maximal inhibitory concentration (IC_{50}) values were determined using a sigmoidal dose−response model from GraphPad Prism v. 4.0 (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

Hyperbranched PEO-*hb*-PG copolymers were prepared according to the modified Frey's method (Scheme 2). Initially, the M_n of the commercially obtained PEO monomethyl ether was determined by GPC in DMF calibrated with polystyrene standard samples, and NMR analysis in deuteriated chloroform. The M_n, polydispersity (M_w/M_n), and the degree of polymerization determined by GPC were 1720 g/mol, 1.20, and 39, respectively, and these results agreed well with those obtained by NMR analysis. The hydroxyl functional group at the terminal of PEO was transformed into the macroinitiator of PEO-OCs for the polymerization of ethoxyethyl glycidol ether (EEGE) by dehydration with cesium hydroxide. After the removal of unreacted PEO by precipitation in cold diethyl ether, the acetal group was cleaved by HCl. After block copolymerization, we found no EEGE was remained in the final mixture by NMR, suggesting the presence of unreacted PEO, because the yield of the purified block copolymer was not quantitative (59%). Thus, the block efficiency was calculated to be 0.39 (see Experimental Section for details). The relatively low block efficiency might be attributed to the heterogeneous macroinitiator in 1,4-dioxane solution. The M_n of the resulting block copolymer PEO_x-*b*-PG_y was measured by GPC to be 3685 g/mol (M_w/M_n = 1.19) (Table 1), which showed a good agreement with the calculated value of 3900 g/mol (see Experimental Section for details). Thus, the block efficiency was calculated to be 0.39. The repeating numbers of each block were determined to be 39 for PEO and 30 for PG.

Table 1. Characterization Data for Polymers Used in This Study

run	polymer[a]	yield (%)	M_n [b]	$M_{n(calc)}$ [c]	M_w/M_n [b]
1	PEO_{39}		1720		1.20
2	PEO_{39}-*b*-PG_{30}	59	3685	3900	1.19
3	PEO_{39}-*hb*-PG_{93}	65	9300	8580	1.25

[a]Commercially available PEO of nominal M_n of 2000 (Aldrich). [b] Determined by GPC ($CHCl_3$, PS standards). [c] Calculated values from the yield of the polymer (see Experimental Section for details).

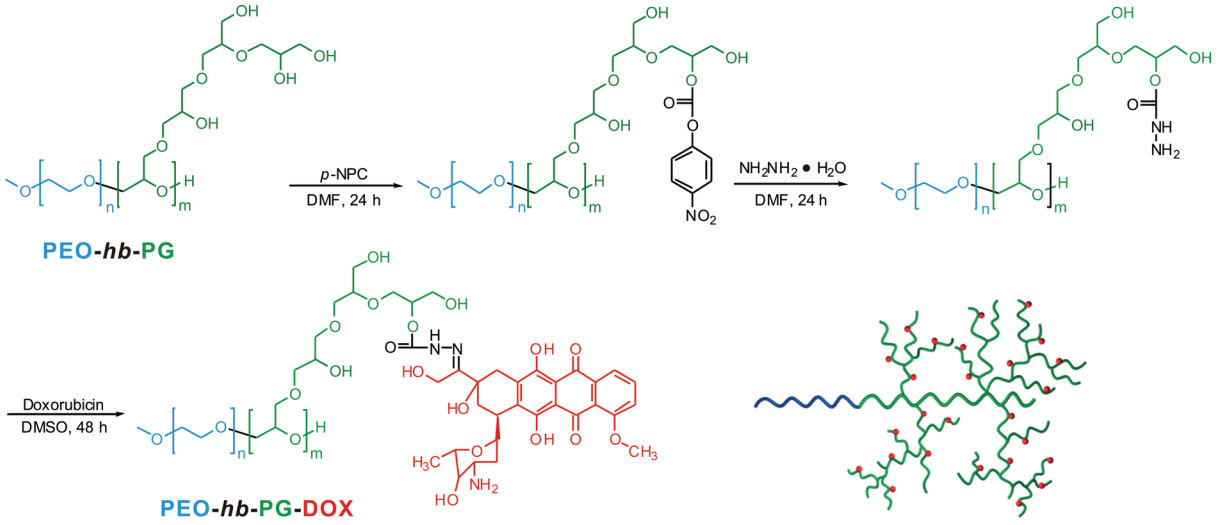
The hypergrafting of linear PEO_{39}-*b*-PG_{30} with glycidol was then performed. About 4.1 equiv of glycidol to OH function on the linear macroinitiator was hypergrafted in solution. As a result, the hypergrafted copolymer was obtained in 65% yield. Imperfect yield might be attributed to the presence of glycidol homopolymer, which can be removed by precipitation in cold diethyl ether. We assumed that the PEO-*hb*-PG was quantitatively collected during the process of repeated precipitation, and therefore, the nonquantitative yield should come from the formation of homo-PG during the hypergrafting stage of polymerization. We verified the above assumption by NMR of the soluble product in cold ether. Again, the M_n of the resulting hypergrafting copolymer PEO_{39}-*hb*-PG_z was measured by GPC to be 9300 g/mol (M_w/M_n = 1.42), which shows good agreement with that from the calculated value 8580 g/mol. The repeated number of z was determined to be 93. Therefore, the number of OH function per a polymer chain can be calculated to be 93.

The conjugation of DOX to the prepared hyperbranched polymer to afford PEO-*hb*-PG-DOX was achieved by a procedure similarly described in a literature.[29] After the preparation of the PEO-*hb*-PG copolymer, the multiple hydroxyl groups on the PG segment were further modified with DOX via an acid-labile hydrazone linkage. The hydroxyl groups were first modified using *p*-NPC mediated hydroxyl amine coupling reaction followed by an amine-ketone reaction with DOX to provide a pH-responsive feature of PEO-*hb*-PG-DOX as shown in Scheme 3. The chemical structure of PEO-*hb*-PG-DOX was characterized with ^{1}H NMR spectra to confirm the presence of the aromatic DOX (See Supporting Information). Moreover, the degree of conjugation was determined to be around 2.1%, on the basis of HPLC measurement.

Once the conjugation of DOX was confirmed, the PEO-*hb*-PG-DOX was dialyzed against the pH-adjusted deionized water to form the micellar structures that consist of the core of hydrophobically modified PG-DOX and hydrophilic PEO shell, which can stabilize the resulting micellar structures. The size of micelles measured by dynamic light scattering (DLS) was 183.3 ± 6.03 nm, confirming the formation of the micelles after to conjugation of DOX, while that of unmodified plain polymer (PEO-*hb*-PG) was 4.91 ± 1.96 nm (Figure 1). This significant difference indicates the successful formation of a core−shell type micellar nanostructure on conjugation with hydrophobic DOX, while plain double hydrophilic block copolymer of PEO-*hb*-PG does not induce any micelle formation. This feature is important because after the delivery of the drug to a site from micelles of PEO-*hb*-PG-DOX, the structures can potentially disassemble to all-biocompatible, double hydrophilic block copolymer of PEO-*hb*-PG, which can be readily cleared and/or biodegraded. According to the study by Brooks and co-workers, the hydrophobically modified PEO containing PG could degrade particularly well under acidic conditions of an intracellular environment.[23] It should also be noted that the hyperbranched morphology of PG has a great potential for developing a promising system for enhanced drug loading.[31]

The morphology of micelles was observed by transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Figure 2). Figure 2a showed the spherical micelles consisting of a dark core (average diameter of 240 nm) and a relatively brighter shell layer (average thickness of 70 nm) in an aqueous solution. The overall average diameter of 310 nm was relatively larger than that obtained from the DLS measurement, which could be attributed to the flattening effect induced during sample preparation. In addition, AFM image in Figure 2b indicates the hydrophobic core is surrounded by hydrophilic shell. The core/shell morphology of the PEO-*hb*-PG-DOX micelle is also promising for cancer therapy, since the biocompatible PEO shell can provide a stealth property with prolonged circulation life while the stabilized internal core can effectively prevent early burst release of encapsulated therapeutics from the micelle during circulation.

Scheme 3. Synthetic Approach for the Formation of Doxorubicin Conjugated PEO-*hb*-PG-DOX



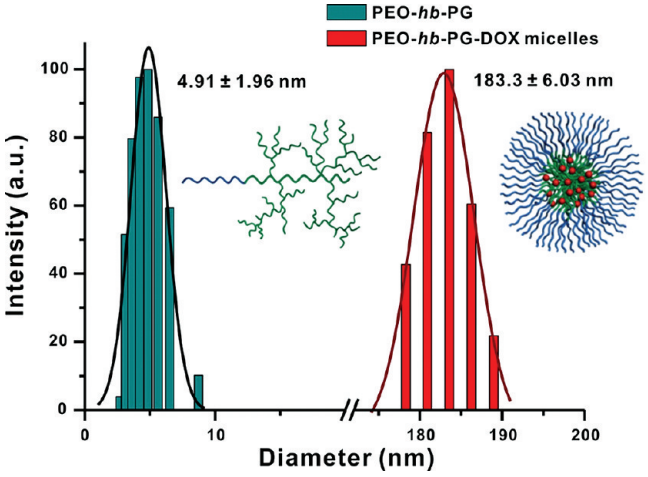


Figure 1. Dynamic light scattering (DLS) analysis of free PEO-*hb*-PG and PEO-*hb*-PG-DOX micelles.

To verify the acid-labile cleavage of the hydrazone bond, the release of DOX from the micelles was studied at pH 5.0 and 7.4 conditions (Figure 3). As expected, PEO-*hb*-PG-DOX micelles exhibited the pH-responsive DOX release profile due to the hydrazone bond linkage between DOX and polymer backbone. The PEO-*hb*-PG-DOX micelles released 57.7% of DOX within 3 h and liberated 71% after 48 h. In contrast, the amount of released DOX was 53.6% after 48 h in the case of samples subjected to pH 7.4. As suggested in other reports, the accelerated release of DOX under acid conditions is desirable for an effective cancer therapy, because intracellular endosomal pH within the tumor cells is considerably lower than that of the normal tissue.[32]

In vitro efficacy of PEO-*hb*-PG-DOX micelles was investigated by cell viability assay using a human cervical cancer HeLa cell line (Figure 4). A modified MTT assay was performed by subjecting release aliquots to HeLa cells to ascertain that DOX retained its activity. As shown in Figure 4, PEO-*hb*-PG-DOX micelles induced significant cytotoxicity in HeLa cells, while virtually no toxicity was observed from PEO-*hb*-PG treated cells at the entire concentration ranges tested. The IC_{50} value of PEO-*hb*-PG-DOX was estimated to be around 6.9 μM of polymer concentration. In addition, it is

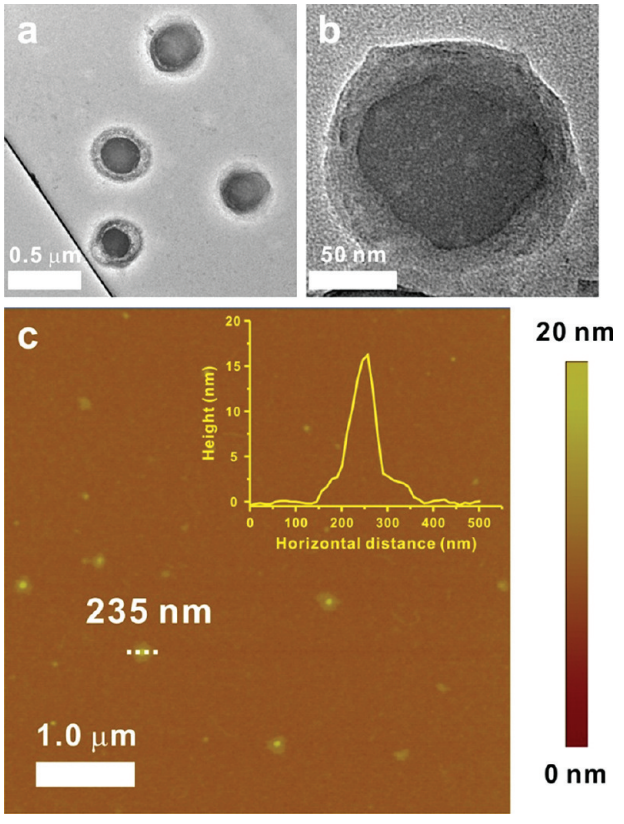


Figure 2. Representative (a, b) TEM and (c) AFM images of PEO-*hb*-PG-DOX micelles. Inset shows the line scan of one of the micelles with an average diameter of 235 nm, which is corresponding to the results from DLS.

evident that the plain double hydrophilic copolymer of PEO-*hb*-PG is highly biocompatible and nontoxic, confirming the desired feature of our system.

The cellular uptake efficiency of PEO-*hb*-PG-DOX micelles was further studied by confocal laser fluorescence microscopy. HeLa cells were also incubated with the same dose of plain PEO-*hb*-PG polymers for 4 h as a control. Figure 5 shows that the clear red fluorescence of DOX appears in the cells treated DOX-loaded micelles, suggesting the successful internalization

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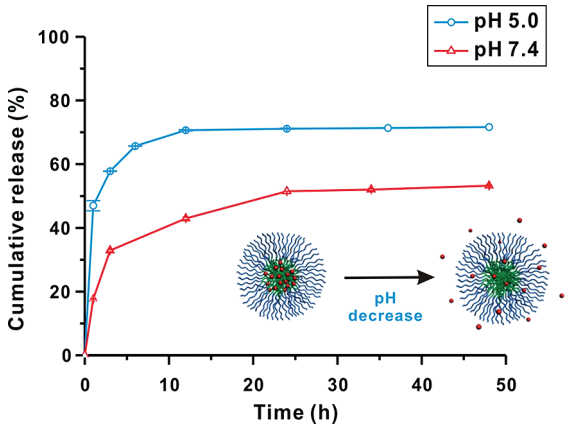

Figure 3. Release profiles of doxorubicin from PEO-*hb*-PG-DOX micelles under different pH conditions. (open circle) pH 5.0 and (open triangle) pH 7.4. The release profile was normalized to the amount of doxorubicin initially loaded into the micelle core.

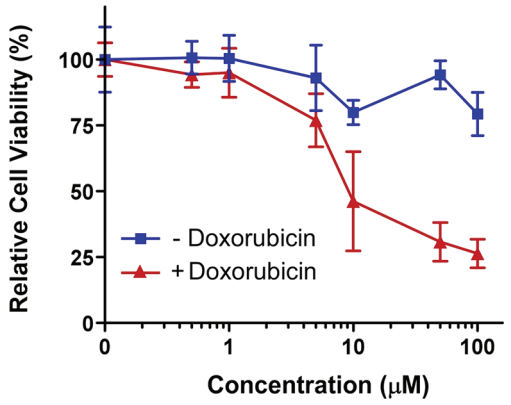

Figure 4. MTT-based cell viability assay of (blue square) plain PEO-*hb*-PG and (red triangle) PEO-*hb*-PG-DOX micelles.

of micelles within the cells. Interestingly, more DOX was accumulated than in the cytosol. According to other report, DOX accumulated only in the nuclei of HeLa cells treated with free DOX, because of the rapid transport of intracellular DOX from the cytosol to the nucleus and avid binding of DOX to chromosomal DNA. In contrast, it appears that the PEO-*hb*-PG-DOX micelles were initially located within the endosomal vesicles, releasing cleaved DOX in a controlled manner.

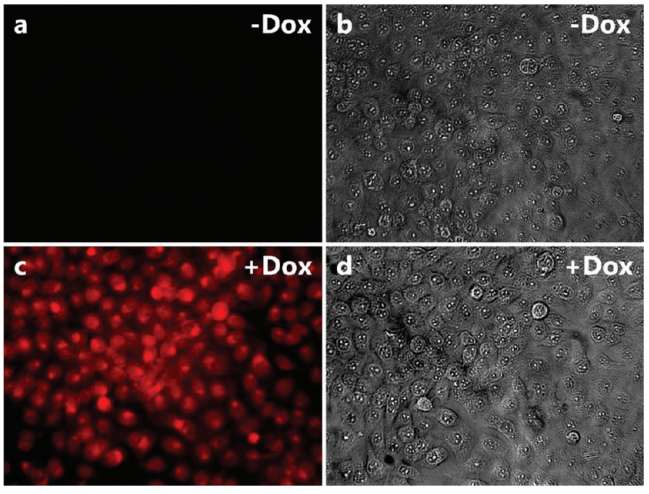

Figure 5. (Left) Confocal fluorescence images of cellular uptake of polymers and (right) corresponding phase-contrast images. (a, b) plain PEO-*hb*-PG and (c, d) PEO-*hb*-PG-DOX micelles. HeLa cells were incubated with micelles at a 20 μM concentration for 4 h.

CONCLUSION

In summary, we designed and synthesized a new class of hyperbranched double hydrophilic block copolymer of poly(ethylene oxide)-*hyperbranched*-polyglycerol (PEO-*hb*-PG) with an aim to enhance biocompatibility, increase water solubility, and improve the clearance of the polymer after delivery of the drug. The model anticancer drug, doxorubicin (DOX), was conjugated to PEO-*hb*-PG via an acid-labile hydrazone linkage. The doxorubicin-conjugated copolymer of PEO-*hb*-PG-DOX underwent a spontaneous self-assembly process in aqueous solution to create core−shell type micellar aggregates. Upon exposure to an acidic condition, the PEO-*hb*-PG-DOX micelles released DOX to a greater extent through the cleavage of acid-labile hydrazone bonds. The cytotoxicity of the PEO-*hb*-PG-DOX micelles was also evaluated with HeLa cells, which showed a marked increase of efficacy with the concentration, whereas the plain PEO-*hb*-PG polymer does not show any noticeable cytotoxicity, confirming the highly biocompatible and nontoxic features of double hydrophilic block copolymer. We believe that this double hydrophilic block copolymer will provide a platform not only to improve the solubility of the drugs in aqueous solution, but also to enhance the targeting capacity to the solid tumor.

ASSOCIATED CONTENT

Supporting Information

Additional ^{1}H and ^{13}C NMR spectra of the polymers are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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