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Poly (ethylene glycol)-armed hyperbranched polyoxetanes for anticancer drug delivery

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Abstract

A facile method for synthesis of polyethylene glycol (PEG)-armed hyperbranched polyoxetanes is presented as well as characterization and use in drug delivery. A series of hyperbranched polyoxetanes with multiple PEG arms were synthesized via a one-pot cationic ring-opening polymerization of 3-ethyl-3-hydroxymethyloxetane (EHMO) and its PEGylated derivative (EPMO), in which the feed mass ratio of EHMO to EPMO was 98:2, 96:4, 74:26, or 17:83. Characterization methods included NMR, DLS, FT-IR, DSC, and SEM. Toxicity of the synthesized polymers to human dermal fibroblasts was evaluated using the MTT assay. Formulation into particles was carried out to encapsulate the anticancer drug camptothecin using the single oil-in-water (o/w) solvent evaporation method. The resulting drug encapsulated particles were evaluated for antitumor activity using HN12 cells.

Keywords

anticancer drug delivery; hyperbranched polymer; PEGylation; polyether polyol

Introduction

Hyperbranched polymers and dendrimers with defined structures have been the subject of extensive research over the past decade and have drawn attention for pharmaceutical applications owing to their unique architectures and high content of reactive end groups.^{1–12} Hyperbranched polymers with a wide range of structural characteristics and properties have been synthesized by different polymerization techniques such as polycondensation,^{1,2} proton-transfer polymerization,^{13,14} and ring-opening polymerization (ROP).^{15–19} The structural regularity of hyperbranched polymers is not as high as dendrimers, but synthesis is less complicated and more cost-effective for scale-up, thus positioning hyperbranched polymers as a promising class of vehicles for drug delivery. In particular, hyperbranched polyether polyols have inherently desirable characteristics for drug delivery because hydrophobic drugs can be encapsulated in the hydrophobic core while hydroxyls at the periphery can be functionalized for conjugation with biological targets.^{6,20–23}

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Poly(3-ethyl-3-(hydroxymethyl) oxetane) (P(EHMO)) is an example of a hyperbranched polyether polyol synthesized by cationic ROP that contains multiple primary hydroxyl groups.^{16–19} PEGylation has been widely implemented to modify hyperbranched polymers, dendrimers, and nanoparticles to render them biocompatible.^{24–26} In addition to the use of branched PEG,²⁷ a shell of PEG arms on the periphery of hyperbranched P(EHMO) can be generated through polymerization of ethylene oxide initiated by surface-accessible hydroxyl groups.²⁸ However, this is usually done by post-polymerization chemical modification and requires peripheral functional groups as anchor points. Inadvertently, post-polymerization PEGylation results in a decrease in the number of functional groups available for drug conjugation or additional functionalization. A strategy to integrate PEG so as to make the carrier biocompatible while maintaining a high proportion of peripheral hydroxyl groups for covalent drug loading is desirable.²⁶

Herein, we report a facile method for synthesis of PEG-armed hyperbranched polyoxetanes via one-pot cationic ROP of EHMO monomer and its PEGylated derivative (EPMO macromonomer). The motivation for incorporating PEG into EHMO prior to polymerization was to reduce synthesis steps for making biocompatible branched polymers as well as to use PEGylated monomer to modulate the structure and properties of the resultant hyperbranched polymers. In this new method, the synthesis of the hyperbranched polyoxetane core by ROP of EHMO was followed by addition of EPMO to produce a hyperbranched macromolecular architecture with PEG arms forming the corona. The synthesis method and characterization of the resultant hyperbranched P(EHMO-EPMO) are reported. In addition, hyperbranched P(EHMO-EPMO) was processed into particles for encapsulation of the anticancer drug camptothecin (CPT) using the single oil-in-water (o/w) solvent evaporation method. The sustained release and antitumor activity of CPT-loaded P(EHMO-EPMO) particles were examined.

Materials and Method

Materials

3-Ethyl 3-(hydroxymethyl) oxetane (EHMO) was received as a gift from Perstorp Polyols (Toledo, OH). Methoxy PEG amines (mPEG-NH₂) with molecular weights of 550 and 2000 g·mol⁻¹ were purchased from Nanocs (New York, NY) and JenKem Technology USA (Allen, TX), respectively. Boron trifluoride diethyl etherate (BF₃·O(C₂H₅)₂), dichloromethane (DCM), dimethylformide (DMF), CPT, chloroform, methanol, 4-nitrophenyl chloroformate (NPC), tetrahydrofuran (THF), and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

Synthesis of EPMO Macromonomer

EPMO macromonomer was synthesized by coupling mPEG-NH₂ to EHMO via the hydroxymethyl side group following a procedure described previously (Scheme 1).²⁹ Briefly, EHMO (2.5 mmol) was dissolved in 5 mL of THF followed by addition of TEA (2.5 mmol) and NPC (2.5 mmol). The mixture was stirred for 24 h and then centrifuged to remove triethylammonium chloride. The supernatant was collected and roto-evaporated to remove the solvent. The residue was further reacted with an equimolar amount of mPEG-NH₂ (*M_n* = 550 or 2000 g·mol⁻¹) in 5 mL of DMF at room temperature. At 72 h, the resultant EPMO macromonomer was dialyzed against deionized water and freeze dried. A 60% yield was obtained.

Synthesis of Hyperbranched Polyoxetanes

Hyperbranched polyoxetanes were synthesized via a one-pot cationic ROP following a previously reported method (Scheme 2).¹⁸ A three-necked round bottom flask was placed on

a heating mantle at 100 °C under a nitrogen purge for 30 min. Subsequently, a temperature of 45 °C was maintained during the reaction. Upon discontinuation of the nitrogen flow, 15 mL of DCM and $\text{BF}_3 \cdot \text{O}(\text{C}_2\text{H}_5)_2$ catalyst were added. Within 5 min, EHMO was added so that the molar ratio of EHMO to $\text{BF}_3 \cdot \text{O}(\text{C}_2\text{H}_5)_2$ was 2:1. After 48 h, EPMO was added followed by stirring for 24 h. The reaction mixture was quenched with ethanol. The resultant hyperbranched P(EHMO-EPMO) polyol was dried under vacuum at 60 °C for 2 d. A series of P(EHMO-EPMO) polymers were synthesized by adjusting weight ratio of EPMO to EHMO (98:2, 96:4, 74:26, and 17:83) (Table 1). EPMO coupled with mPEG 2000 was used for the synthesis of P(EHMO-EPMO)98:2, P(EHMO-EPMO)96:4 and P(EHMO-EPMO)74:26, whereas EPMO coupled with mPEG 550 was used for the synthesis of P(EHMO-EPMO)17:83. The yield of the synthesized polymers ranged between 50–66%.

Preparation of Drug-loaded Particles

The single o/w solvent evaporation method was used to prepare drug-loaded particles based on P(EHMO-EPMO) polymers.²⁸ Camptothecin (CPT), an anti-cancer drug, was used as a model drug. Drug solutions were prepared by dissolving 10 mg of CPT in 1 mL of chloroform/methanol (0.75 mL/0.25 mL). P(EHMO-EPMO) (10 mg) was dissolved in 3 mL of chloroform/deionized water (1 mL/2 mL). Drug-loaded particles were prepared by combining the drug and polymer solutions. The mixture was kept at room temperature overnight and then dried under vacuum. The dry residue was resuspended in deionized water and subjected to centrifugation at 13,200 rpm for 1 h to remove unencapsulated CPT. The precipitate was dried under vacuum to obtain drug-loaded particles. Particles without drug were also prepared following the same method. The actual amount of CPT encapsulated by the particles was quantified using UV-visible spectrophotometry at 369 nm in chloroform. Encapsulation efficiency (EE) and loading efficiency (LE) were determined according to the

following equations: $EE = \frac{W_1}{W_a}$ and $LE = \frac{W_1}{W_b}$, where W_1 is amount of encapsulated CPT, W_a is total amount of CPT used, and W_b is total amount of particles used. The measurements were repeated in triplicate.

Structure Characterization

Nuclear Magnetic Resonance (NMR) Spectroscopy— ^{13}C -NMR spectra were obtained on a 400 MHz Bruker NMR instrument. Degree of branching (DB) of the resultant hyperbranched polymers was calculated according to the following equation:

$DB(\%) = \frac{D+T}{D+L+T} \times 100$, where D, L, T represent the carbon atoms in dendritic, linear and the terminal units of the hyperbranched polymer.² The corresponding integrals of D, L and T were obtained from their respective peaks centered at 43 ppm.

Fourier Transform Infrared (FT-IR) Spectroscopy—FT-IR spectra of P(EHMO-EPMO) were obtained from solution-cast films on KBr discs using a Nicolet Magna IR 760 spectrometer.

Differential Scanning Calorimetry (DSC)—Thermal analysis measurements were carried out in a nitrogen atmosphere using TA-Q Series Q1000 DSC (TA Instruments, New Castle, DE). Samples (5–6 mg) were heated and cooled between –90 to 120 °C at 10 °C/min. Data from the second heating cycle are reported in Table 1.

Dynamic Light Scattering (DLS) Measurement—A Malvern Zetasizer Nano S (Malvern Instruments, Malvern, UK) was used to determine molecular weights of the synthesized polymers and particle sizes. Polymer samples were suspended in chloroform and filtered through a 2 µm syringe filter prior to measurement. Light scattering intensity

was recorded and scatter intensities were plotted as Zimm plots to calculate the mean particle size and size distribution (i.e., polydispersity index with a value between 0 and 1).

Scanning Electron Microscopy (SEM)—SEM images were taken under a scanning electron microscope ZESIS EVO50. Polymer samples were dissolved in chloroform. A few drops of the solution were placed on a silicon wafer and dried under vacuum. The sample was then mounted on the stub and sputter coated with gold. Images were taken at 35000× magnification.

In Vitro Drug Release Studies

Drug-loaded particles were weighed and suspended in 10 mL of phosphate-buffered saline (PBS) pH 7.4 in a capped conical flask. The suspension was maintained at 37 °C. At predetermined intervals, 3 mL of supernatant was collected and UV-Visible absorbance measurements at 369 nm were used to quantify the released drug. After each measurement, 3 mL of fresh PBS was added back to the medium to continue the release study. The experiment was repeated in triplicate.

Cytotoxicity Assessment

The cytotoxicity of P(EHMO-EPMO) polymers to human dermal fibroblasts was evaluated. Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere containing 10% CO₂ and 90% air. The cells were incubated with polymers at different final concentrations (10, 50, 100, and 300 µg/mL) for 48 hours. The cell viability was assessed using the MTT assay, whereas untreated cells were used as control.

In Vitro Antitumor Activity of CPT-loaded in P(EHMO-EPMO) Particles

The HN12 cells used to examine the in vitro antitumor activity of particles were derived from a lymph node metastasis in a patient.³⁰ HN12 cells were cultured in DMEM supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 10% CO₂ and 90% air. The viability of HN12 cells incubated with drug-loaded particles based on P(EHMO-EPMO)74:26 at a final concentration of 10 µg/mL for various lengths of time (i.e., 12, 24, 48, and 72 h) was determined using the MTT assay. HN12 cells incubated with blank particles of P(EHMO-EPMO)74:26 at the concentration of 10 µg/mL was also assessed.

Results

Characterization of Hyperbranched P(EHMO-EPMO)s

Prior studies have shown that EHMO monomers form branched polymeric structures through cationic ROP using BF₃·O(C₂H₅)₂ catalysis.^{17,18,28} In this work, we employed a two-step one-pot polymerization process to construct a hyperbranched core of P(EHMO) and a shell of PEG arms from P(EPMO). ¹³C-NMR spectroscopy confirmed the synthesis of P(EHMO-EPMO) copolymers and their branched structures. With reference to Scheme 2 for carbon atom designations, in the ¹³C-NMR spectrum of P(EHMO-EPMO)98:2 (Figure 1), peak **a** corresponds to $-\underline{\text{CH}}_3$ in ethyl group, peak **b** corresponds to $-\underline{\text{CH}}_2-$ in ethyl group, peak **c** corresponds to $-\underline{\text{CH}}_2\text{OH}$ group, peak **d** corresponds to $-\underline{\text{CH}}_2-\text{O}-$, peaks **e** and **f** correspond to the repeat unit of PEG (i.e., $-(\underline{\text{CH}}_2-\underline{\text{CH}}_2-\text{O})_n-$), peak **g** corresponds to the PEG terminal methoxy group ($-\text{O}-\underline{\text{CH}}_3$). Three peaks (**D**+**L**+**T**) centered at 43 ppm indicate the presence of dendritic (**D**), linear (**L**), and terminal (**T**) carbons in the 2-position of the resultant polyoxetane hyperbranched structures. These **D**, **L**, and **T** peaks were used to determine degree of branching (DB) of the resulting branched polymer. Incorporation of

EPMO into the resulting hyperbranched polymer was confirmed by ^1H NMR spectrum (not shown).

DB is a key parameter determining properties of hyperbranched polymers. As summarized in Table 1, DB of P(EHMO-EPMO) increases with increasing EP MO wt% in the reaction. As expected for P(EHMO-EPMO) polymers based on EP MO/mPEG 2000, increasing the amount of EP MO macromonomer results in an increase in molecular weight for copolymers. P(EHMO-EPMO)17:83 has a molecular weight of $7.8 \times 10^3 \text{ g mol}^{-1}$, which is the lowest among the synthesized polymers. The use of EP MO/mPEG 550 partly accounts for this observation.

FT-IR spectroscopy was used for a qualitative characterization of functional groups. As shown in Figure 2, P(EHMO-EPMO) generally displays a strong peak at 2880 cm^{-1} due to C-H stretching, a peak at 1110 cm^{-1} due to C-O stretching and a peak at 1740 cm^{-1} due to C=O stretching. As the number of C-O bonds increases due to the incorporation of PEG chains, the absorption peak at 1110 cm^{-1} becomes broader.

DSC was conducted to elucidate thermal behavior of branched P(EHMO-EPMO)s and controls (Table 1 and Table S1). The core P(EHMO) shows an endotherm in the first cycle at 43°C (Figure 3). Considering the high density of peripheral hydroxyl groups, the transition is assigned to a T_g due to the formation of a hydrogen bonded glass during solidification from solution. This endotherm may be partly due to volumetric relaxation as on the second heating cycle T_g is at 27°C (Figure 4). Given that the thermal behavior of EHMO was not reported previously, additional studies are warranted to confirm these preliminary results.

Substitution of hydroxyl groups in the P(EHMO) core with polymerized EP MO resulted in a decrease of T_g for EHMO/EP MO copolymers. Remarkably, only 2 wt% of EP MO (2000Da) results in the disappearance of the core T_g and the appearance of a new T_g at -34°C (Figure 4). Increasing the wt% of EP MO (2000Da) to 4% and 26% results in a small melting endotherm at $50\text{--}55^\circ\text{C}$ during the first heating cycle (Figure 3). Given that linear mPEG2000-NH₂ (Figure S2) has a T_m at 52°C , the small melting endotherms for the 4 and 26 wt% compositions may be due to T_m for a crystalline phase formed by the EP MO arms. These melting transitions were not observed in the second cycle (Figure 4). Regardless of heating cycle, T_g values for 4 and 26 wt% EP MO compositions were -21 and -25°C , respectively. P(EHMO-EPMO)17:83 has the highest EP MO composition, which was constructed on the basis of mPEG550-NH₂. Unlike its linear control mPEG550-NH₂ (Figure S3) that shows a T_m at 12°C , P(EHMO-EPMO)17:83 only has a T_g at -55°C . Despite a high EP MO content, the EHMO core apparently inhibits crystallization. The lower T_g suggests a higher chain mobility for shorter P(EP MO) arms.

Characterization of Drug-loaded P(EHMO-EPMO) Particles

P(EHMO-EPMO) and CPT-loaded P(EHMO-EPMO) particles were prepared by using the single o/w solvent evaporation method, and their morphology and size were characterized using SEM and DLS, respectively. According to DLS measurements, the diameter of P(EHMO-EPMO) particles ranged from 361 nm to 1078 nm. SEM images (Figure 5) show that the particles were mostly spherical before drug encapsulation and became oval after drug encapsulation. Furthermore, the size of the drug-loaded particles was much larger than that of the blank particles. The size increase and shape change are attributed to drug encapsulation as well as a change in event of aggregation of particles in the presence of drug. Drug loading efficiency and particle encapsulation efficiency were generally high, ranging from 60% to 80% (Table 2). Drug loss during drug loading into particles was due to

resuspension of drug-loaded particles in water and subsequent centrifugation, causing removal of released or unencapsulated CPT.

Cytotoxicity of hyperbranched P(EHMO-EPMO)s

The cytotoxicity of hyperbranched P(EHMO-EPMO) polymers to human dermal fibroblasts was examined. The MTT assay results clearly indicate that cytotoxicity is dependent on dose and EP MO composition (Figure 6). Among the range of the doses from 10 to 300 µg/mL, P(EHMO-EPMO)74:26 and P(EHMO-EPMO)17:83 are more cytocompatible than P(EHMO-EPMO)98:2 and P(EHMO-EPMO)96:4. Only 26.7% cells remained viable after being treated with 10 µg/mL P(EHMO-EPMO)98:2 for 48 h, and the cell viability dropped to 4.4% when the concentration of P(EHMO-EPMO)98:2 increased to 300 µg/mL. A marginal increase in percentage of EP MO did not improve the cytocompatibility of the hyperbranched polymer. P(EHMO-EPMO)96:4 displays a similar level of dose-dependent toxicity as compared to P(EHMO-EPMO)98:2. However, the cytocompatibility of P(EHMO-EPMO) was significantly enhanced as the EP MO composition was increased to 26 wt% or 83 wt%. The viability of HN12 cells was 55.1% in the presence of 300 µg/mL P(EHMO-EPMO)17:83. The cells remained viable as high as 86.6% as the concentration of P(EHMO-EPMO)17:83 was reduced to 10 µg/mL. The cytocompatibility of P(EHMO-EPMO)74:26 is similar to that of P(EHMO-EPMO)17:83 although it has a lower percentage of EP MO in the branched structure than P(EHMO-EPMO)17:83. Using P(EHMO-EPMO)74:26 as an example (Figure 7), the cell morphology observed was consistent with the cell viability assay. As the dose of P(EHMO-EPMO)74:26 increased, more cells became rounded, detached from the substrate, or lysed, indicating cell viability loss. In general, a cell viability of 80% or higher indicates good cytocompatibility of the tested polymers at dosage levels (10 µg/mL or lower), which can be used for in vivo testing.

Drug Release and Antitumor Effect of CPT Following Release

CPT has poor aqueous solubility and antitumor activity can be impaired due to hydrolysis, and conversion from a therapeutically active lactone form to an inactive carboxylate form. Encapsulating CPT into particles helps increase CPT stability and sustain its antitumor activity.^{31,32} As shown in Figure 8, the release kinetics of CPT from P(EHMO-EPMO) particles follows a similar pattern regardless of P(EHMO-EPMO) composition. Almost 100% of the encapsulated drug was released over 9 d. An initial burst release of CPT from P(EHMO-EPMO) particles occurred in the first five hours, followed by a sustained slower release. Since CPT is hydrophobic, a hydrophobic interaction between the drug and the hydrophobic core of P(EHMO-EPMO) is presumed responsible for the sustained drug release.

The antitumor effect on HN12 cells, derived from metastatic squamous cell carcinoma, was evaluated for P(EHMO-EPMO)74:26 drug-loaded particles. HN12 cells were cultured in the presence of 10 µg/mL drug-loaded P(EHMO-EPMO)74:26 particles for 12 h, 24 h, 48 h, or 72 h. The cellular enzymatic activity was quantified with the MTT assay. HN12 cells treated with blank P(EHMO-EPMO)74:26 particles under the same condition were also assessed to study the effect of particles alone on the cells. A marked decrease in the cellular activity of HN12 cells was observed as the incubation with drug-loaded particles was extended from 12 h to 72 h (Figure 9). Cell viability was reduced to 20% by 12 h-incubation of drug-loaded particles and further reduced to 1% by 72 h incubation. This study suggests that longer incubation along with sustained release of CPT results in higher toxicity for HN12 cells. The in vitro release studies showed that 50% encapsulated CPT was released from P(EHMO-EPMO)74:26 particles in 56 h and complete drug release took over 200 h. Taken together, it is expected that the sustained release beyond 72 h would result in complete cell death.

Although the dose of drug-loaded particles remains to be optimized, the above studies demonstrate high potency of the encapsulated drug due to sustained release.

Interestingly, cellular activity of HN12 cells was reduced initially as measured at 12 h post-incubation with blank P(EHMO-EPMO)74:26 particles but recovered following longer incubations. For example, 81% cell viability remained at 72 h post-treatment, indicating the adjustment of the cells to the addition of particles and the relatively low toxicity of particles themselves to the cells.

Discussion

Drug delivery carriers are commonly modified with PEG to gain favorable biological compatibility. PEGylation of drug carriers can be accomplished in two ways: directly grafting PEG to the carrier or forming PEG arms through polymerization of ethylene oxide. To efficiently couple PEG to the branched structures and keep the degree of PEGylation as low as possible, we explored a new route to synthesize PEGylated polyether polyols via a two-step cationic ring-opening copolymerization of EHMO and PEGylated EHMO (i.e. EPMO). In our reaction scheme, a hyperbranched polyether P(EHMO) core was formed by ring-opening polymerization of EHMO, driven by the nucleophilic attack of either the oxygen atom in the ring or the hydroxyl group of EHMO. Since the hydroxyl group of EPMO was substituted with PEG, the reaction was continued by the nucleophilic attack of the oxetane oxygen atom of EPMO. As a result, some new branches with one PEG chain on each branch were generated.

To understand how EPMO affects the resultant polymers, the mass ratio of EHMO to EPMO in polymerization was varied from 98:2 to 17:83. The resulting hyperbranched polyether polyols were found to have a range of properties and structural characteristics including molecular weight, degree of branching, and glass transition temperature. The PEGylated carrier became more cytocompatible and water soluble as more EPMO was included. Our studies confirmed that incorporation of PEG into EHMO prior to polymerization limited PEGylation to a small portion of the branches, while at the same time enabling improvement of the cyto-compatibility for the carrier. Mechanistic understanding of polymerization with the participation of PEGylated monomer will be sought in future studies for improved nano- and microscale control of such structures. In addition, multiple hydroxyl end groups in P(EHMO-EPMO) can be utilized for covalently coupling drugs or other functional moieties of interest. Our future studies will include optimization and biofunctionalization of P(EHMO-EPMO) for efficient drug delivery.

Conclusion

A series of core-shell amphiphilic hyperbranched P(EHMO-EPMO) polymers were prepared via a one-pot sequential cationic ring-opening co-polymerization of EHMO and its PEGylated derivative. The structure characteristics and properties of the hyperbranched copolyoxetanes such as degree of branching, molecular weight, and glass transition temperature were varied by changing the mass ratio of EHMO to EPMO. P(EHMO-EPMO) polymers became more cytocompatible with the increase in the percentage of EPMO. Particles for delivery of CPT were formulated from core-shell, hyperbranched P(EHMO-EPMO) copolyoxetanes and the anticancer drug camptothecin (CPT) using the single o/w solvent evaporation method. Sustained in vitro release of CPT over a period of 9 d was achieved. The encapsulated CPT exerted antitumor activity upon release from P(EHMO-EPMO) particles. In summary, these studies showed that hyperbranched P(EHMO-EPMO) can be engineered to possess biologically favorable properties and formulated for delivery of water-insoluble anticancer drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 ^{13}C -NMR spectrum of P(EHMO-EPMO)98:2 (A) and the three peaks around 43 ppm used for calculation of DB (B). Peaks are designated according to Scheme 2.

Figure 2.
FT-IR spectra of P(EHMO-EPMO)s.

Figure 3.
DSC thermograms (1st heating) of P(EHMO) and P(EHMO-EPMO)s.

Figure 4.
DSC thermograms (2nd heating) of P(EHMO) and P(EHMO-EPMO)s.

Figure 5.

SEM images of blank particles (column 1) and CPT-loaded particles (column 2) based on P(EHMO-EPMO)98:2 (A), P(EHMO-EPMO)96:4 (B), P(EHMO-EPMO)74:26, and P(EHMO-EPMO)17:83 (D). Particles were prepared using the single o/w solvent evaporation method. Scale bars: A1: 200 nm; B1: 500 nm; C1: 200 nm; D1: 2 μ m; A2, B2, and C2: 500 nm; and D2: 5 μ m.

Figure 6.

Cytotoxicity of P(EHMO-EPMO) polymers to human dermal fibroblasts. The cells were incubated with the polymer at the indicated concentrations for 48 h and assessed with the MTT assay.

Figure 7.
Phase-contrast images of human dermal fibroblasts treated with P(EHMO-EPMO)74:26 at 10 µg/mL (A), 50 µg/mL (B), 100 µg/mL (C), and 300 µg/mL (D) for 48 h.

Figure 8.
Drug release kinetics of CPT from drug-loaded particles prepared from P(EHMO-EPMO)s.

Figure 9.

Antitumor effect of CPT-loaded P(EHMO-EPMO)74:26 particles on HN12 cells. The cells were incubated with CPT-loaded particles for various lengths of time and assessed with the MTT assay.

Scheme 1.
Synthesis of EPMO macromonomer.

Scheme 2.

Synthesis of hyperbranched P(EHMO-EPMO)s via one-pot cationic ring-opening polymerization.

Table 1

Characteristics of hyperbranched P(EHMO-EPMO)s

Polymer	DB (%)	MW (10^3g mol^{-1})	T_g ($^{\circ}\text{C}$)
P(EHMO-EPMO)98: 2	17.9	21.0	−34
P(EHMO-EPMO)96: 4	48.5	28.1	−21
P(EHMO-EPMO)74: 26	76.4	34.3	−25
P(EHMO-EPMO)17:83	78.0	7.8	−55

Table 2

Characteristics of P(EHMO-EPMO) particles prepared by the single o/w solvent evaporation method

Polymer used to make particles	Particle Size (nm)	PDI	EE (%)	LE (%)
P(EHMO-EPMO)98: 2	686±5	0.95	66	80
P(EHMO-EPMO)96: 4	509±6	1.00	65	74
P(EHMO-EPMO)74: 26	361±7	1.00	64	65
P(EHMO-EPMO)17:83	1078±6	0.88	60	66