Synthesis and Characterization of Degradable Hyperbranched Poly(2-ethyl-2-oxazoline)

Muhammad Waqas Ali,[1] Siyuan Cheng,[1] Jianhao Si,[1] Muhammad Siddiq,[2] Xiaodong Ye [1,3]

[1]Hefei National Laboratory for Physical Sciences at the Microscale, Department of Chemical Physics, University of Science and Technology of China, Hefei, Anhui 230026, China
[2]Department of Chemistry, Quaid-I-Azam University, Islamabad 45320, Pakistan
[3]CAS Key Laboratory of Soft Matter Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China
Correspondence to: X. Ye (E-mail: xdye@ustc.edu.cn)

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ABSTRACT: Hyperbranched poly(2-ethyl-2-oxazoline) was synthesized by a combination of cationic ring-opening polymerization and the oxidation of thiol to disulfide groups. A three-arm star poly(2-ethyl-2-oxazoline) (PEtOx) was first synthesized using 1,3,5-tris(bromomethyl) benzene as an initiator. The star PEtOx was end-capped with potassium ethyl xanthate. Similarly, a linear PEtOx was synthesized and end-capped with potassium ethyl xanthate using benzyl bromide as an initiator. Hyperbranched PEtOx was then obtained by *in situ* cleaving and subsequent oxidation of the star PEtOx and linear PEtOx mixture with *n*-butylamine as both a cleaving agent and a base in tetrahydrofuran. The linear PEtOx was used to prevent the formation of gel. The hyperbranched PEtOx can be cleaved with dithiothreitol to trithiol and monothiol polymer. The hyperbranched PEtOx shows no remaining thiols using Ellman's assay. The resulting hyperbranched PEtOx was hydrolyzed to a novel hyperbranched polyethyleneimine with degradable disulfide linkages. © 2019 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2019**, *57*, 2030–2037

KEYWORDS: degradation; gelation; hyperbranched; ring-opening polymerization; starpolymers

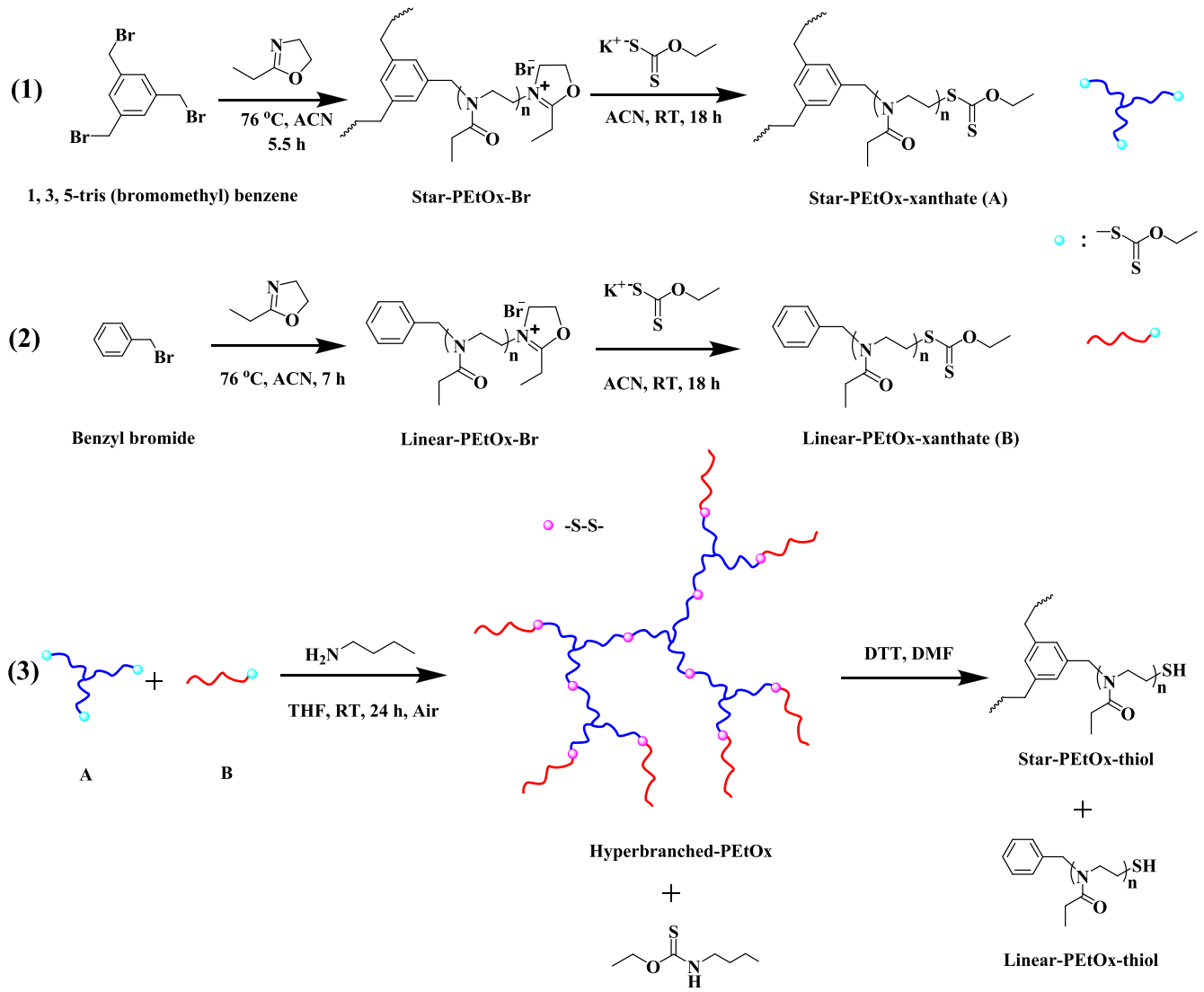
INTRODUCTION Poly(2-oxazoline)s (POx) are a promising class of polymers for material science applications.[1–9] The solution properties of POx strongly depend on the hydrocarbon side chains. For example, poly(2-methyl-2-oxazoline) is water soluble in the temperature region of 0–100 °C. Poly(2-ethyl-2-oxazoline) and poly(2-isopropyl-2-oxazoline) have cloud point temperatures of ~60 and ~35 °C.[5,10–13] Poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) can be used as alternatives to poly(ethylene oxide) due to the water-soluble and antifouling properties.[4,14,15] Besides, linear POx, POx with different topologies, such as cyclic,[16–19] star-branched,[20–24] and hyperstar[25] polymers, have been prepared. However, hyperbranched POx has been seldom synthesized. For example, Perrier et al. recently synthesized hyperbranched poly(2-ethyl-2-oxazoline) using thiol-yne chemistry and then obtained hyperbranched poly(ethylenimine-co-oxazoline) after hydrolysis.[26] They found that the hyperbranched poly(ethylenimine-co-oxazoline) polymer has slightly lower transfection efficiencies and reduced toxicity compared to the commercial standard branched polyethylenimine with a molecular weight of 25,000 g/mol. However, this hyperbranched polymer is nondegradable, which may restrict its application. In this study, we synthesized a degradable hyperbranched poly(2-ethyl-2-oxazoline) by oxidation of thiol groups to disulfide bonds, as shown in Scheme 1, since the main advantage of the disulfide bond is that it can be cleaved to thiol groups using various reducing agents.[27–31] Briefly, a three-arm star PEtOx end-capped with potassium ethyl xanthate (Star-PEtOx-xanthate) was first synthesized by a trifunctional initiator. A linear PEtOx with potassium ethyl xanthate (Linear-PEtOx-xanthate) was also prepared. Hyperbranched PEtOx was then formed by subsequent cleavage and oxidation of a mixture of star-PEtOx-xanthate and linear-PEtOx-xanthate. The hyperbranched polymer was characterized extensively to confirm the structure.

EXPERIMENTAL

Materials

Acetonitrile (ACN, Sinopharm, 99%) was first stirred with potassium permanganate and distilled. The resulting solvent was stirred with calcium hydride (CaH_2) and freshly distilled before use. 2-ethyl-2-oxazoline (Alfa, 99%) was fractionally distilled from CaH_2 prior to polymerization. *N*,*N*-dimethylformamide (DMF, Sinopharm, 99%) was dried from magnesium sulfate and fractionally distilled under a reduced pressure. Tetrahydrofuran (THF, Sinopharm, 99%) was refluxed over sodium for 24 h and

Additional supporting information may be found in the online version of this article.



SCHEME 1 Schematic representation of the synthesis of the degradable hyperbranched poly(2-ethyl-2-oxazoline). Step 1 and Step 2 represent the synthesis of star-PEtOx-xanthate and linear-PEtOx-xanthate, respectively. Step 3 shows the synthesis of degradable hyperbranched PEtOx by the cleavage of the linear and star polymers and subsequent air oxidation. [Color figure can be viewed at wileyonlinelibrary.com]

distilled prior to use. The salt potassium ethyl xanthate (Aladdin, 98%) was purified by recrystallization from acetone/petroleum ether. Milli-Q water with a resistivity of 18.2 MΩ cm was used. All the other reagents were used without further purification.

Synthesis of Three-Arm Star PEtOx (Star-PEtOx-Br)

A trifunctional initiator 1,3,5-tris (bromomethyl) benzene (0.25 g, 0.70 mmol), the monomer 2-ethyl-2-oxazoline (2.08 g, 21 mmol), and 6.0 mL of ACN were charged to a pre-dried polymerization tube. The solution was degassed using three freeze-pump-thaw cycles and the tube was sealed. The polymerization reaction was carried out at 76 °C for 5.5 h. The three-arm star poly(2-ethyl-2-oxazoline) was precipitated three times in ice-cold diethyl ether.

End-Capping of Living Three-Arm Star PEtOx with Potassium Ethyl Xanthate (Star-PEtOx-Xanthate)

Potassium ethyl xanthate (0.64 g, 4.0 mmol) and 30 mL of ACN were added to a three-neck round-bottom flask with N_2 inlet and outlet. The living polymer solution (2.00 g of polymer, 0.67 mmol) was added to the above mixture under N_2 atmosphere and the reaction was allowed to proceed at room temperature for 18 h. The reaction mixture was filtered to remove the salt and the solvent was evaporated under a reduced pressure using a rotary evaporator. The residue was dissolved in dichloromethane (DCM) and the solution was filtered again. The polymer was washed with aqueous saturated sodium chloride solution (3 × 25 mL). The organic phase was dried with anhydrous sodium sulfate. The salt was removed by filtration and the solvent was removed

using a rotary evaporator. The polymer was precipitated three times in diethyl ether and dried in a vacuum oven at room temperature for 24 h.

Synthesis of Linear Poly(2-Ethyl-2-Oxazoline)

A monofunctional initiator benzyl bromide (0.5 g, 2.92 mmol), 2-ethyl-2-oxazoline (2.90 g, 29.30 mmol), and 9 mL of ACN were added to a pre-dried tube. The system was degassed using three freeze-pump-thaw cycles and the tube was sealed. The polymerization reaction was allowed to proceed at 76 °C for 7 h. The polymer was precipitated in ice-cold diethyl ether three times and dried in a vacuum oven for 24 h at room temperature.

End-Capping of Living Linear PEtOx with Potassium Ethyl Xanthate (Linear-PEtOx-Xanthate)

The living polymer solution was also end-capped with potassium ethyl xanthate. Briefly, 30 mL of the solvent ACN and the salt potassium ethyl xanthate (0.96 g, 6.0 mmol) were added to a round-bottom flask with N_2 inlet and outlet. Then living linear poly(2-ethyl-2-oxazoline) (2.90 g of polymer, 2.9 mmol) ACN solution was added into the above solution. The reaction was stirred at room temperature for 18 h. The product was purified by filtration, the solvent was removed under a reduced pressure, and the residue was dissolved in DCM. The solution was filtered and washed with saturated aqueous sodium chloride solution (3 × 25 mL). The organic phase was dried using anhydrous sodium sulfate and the polymer was precipitated three times in diethyl ether.

Synthesis of Hyperbranched PEtOx

The star-PEtOx-xanthate and linear-PEtOx-xanthate polymers were cleaved and oxidized simultaneously to form hyperbranched PEtOx. Briefly, star-PEtOx-xanthate (1 g, 1.0 mmol of xanthate end group) and linear-PEtOx-xanthate (0.25 g, 0.25 mmol of xanthate end group) were dissolved in 6 mL of THF containing *n*-butylamine (219.0 mg, 3.0 mmol). The reaction was carried out at room temperature for 24 h. The resulting hyperbranched polymer was precipitated from 10-fold excessive diethyl ether and dried in a vacuum oven at room temperature for overnight.

Reduction of Hyperbranched PEtOx

Hyperbranched PEtOx (0.05 g) was dissolved in 2.0 mL of DMF and the solution was degassed by bubbling N_2 for 30 min. Dithiothreitol (DTT) (57.8 mg, 0.374 mmol) was added into the above PEtOx solution and the reaction was carried out at room temperature for 6 h. The solvent was removed under a reduced pressure. The residue was dissolved in chloroform and the polymer solution was precipitated from ice-cold diethyl ether. The polymer was dried in a vacuum oven at room temperature for 24 h.

Hydrolysis of PEtOx to Polyethyleneimine

Star-PEtOx-Br (0.20 g, 0.067 mmol) was dissolved in 50 mL of 1 M hydrochloric acid aqueous solution and the reaction was continued at 100 °C for overnight. The solvent was removed under a reduced pressure and the residue was dissolved in 20 mL of water. 1 M sodium hydroxide solution was added dropwise to the PEI solution until precipitation. The polymer was washed three times with water and then freeze-dried.

Determination of Free Thiol Groups by Spectrophotometry Using Ellman's Reagent

The thiol (—SH) content in the star, linear, and hyperbranched PEtOx was determined using Ellman's assay.[32] Briefly, freshly reduced PEtOx-thiol (5.0 mg, 1.25 μmol) was dissolved in 1 mL deionized distilled water. Fifty microliters of PEtOx-thiol polymer solution with a concentration of 5.0 mg/mL (pH 4, nitrogen bubbled) was added to a mixture of 50 μL of 0.01 mM Ellman's reagent (in 0.1 M phosphate buffer, pH 8.0), 500 μL of 0.1 M phosphate buffer (pH 8), and 450 μL of distilled water. The absorbance of the resulting solution was measured at 412 nm after 5 min. The content of the thiol in the polymers was determined using the molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

Cell Viability Assay

HeLa cells were seeded in 96-well plates at a density of 5000 cells/well in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% antibiotics for 24 h. The medium was replaced with different concentrations of the polymer in DMEM. Then the HeLa cells were incubated for 24 h in 5% CO_2 at 37 °C. DMEM containing the polymer solution was replaced with MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5.0 mg/mL in PBS) and incubated for 4 h. After the desired incubation time, the medium was replaced by DMSO (150 μL) and the absorbance was measured at 595–655 nm.

Characterization

All the ^{1}H NMR spectra were obtained on a 400 MHz Bruker AV-400 spectrometer using deuterated chloroform ($CDCl_3$) as a solvent and tetramethylsilane as an internal standard. The dispersity (M_w/M_n), the number average (M_n), and weight average molar masses (M_w) were determined with a SEC (Waters 1515) equipped with a refractive index (RI, Wyatt WREX-02) and a multiangle laser light scattering (MALLS) detector (Wyatt DAWN EOS). Three styragel columns (HR2, HR4, and HR6) with the oven temperature of the column set at 35 °C and THF as a mobile phase with a flow rate of 1.0 mL/min were used for the fractionation of the polymers with narrow distributed polystyrenes as standards.

RESULTS AND DISCUSSION

Scheme 1 represents the synthesis of the hyperbranched PEtOx. The role of the initiator in the polymerization of polyoxazolines is very important. An efficient initiator leads to a well-defined polymer with high end-group functionality. Here, we chose 1,3,5-tris(bromomethyl) benzene as a trifunctional initiator because the initiator is efficient for the polymerization of 2-ethyl-2-oxazoline and a clean polymerization takes place, and the initiator can be consumed completely.[33,34] Note that previous studies show that both the electrophilicity and

the steric hindrance of initiators can influence the efficiency of the cationic ring-opening polymerization.[35,36]

In the first step, a trifunctional initiator 1,3,5-tris(bromomethyl) benzene was used to synthesize a three-arm star PEtOx. The initiator was efficient and the reaction attained ~100% conversion in 5.5 h, as shown in Figure 1. Figure S1 summarizes the polymerization kinetics of 2-ethyl-2-oxazoline using ^{1}H NMR. A linear increase of $\ln([M]_0/[M])$ is observed (Fig. S2), indicating a fast initiation and slow propagation. Previous studies also support this result. For example, Cai and Litt[20] used the same initiator but a different solvent 1,2-dichlorobenzene and the reaction was completed in 2.5 h at 110 °C. Similarly, Chujo et al.[21] used the same initiator but they replaced the bromide *in situ* by iodine or tosylates and the system was also a fast initiation and slow propagation system.

Figure 2(a) represents the ^{1}H NMR spectra of three-arm star PEtOx (star-PEtOx-Br). The three aromatic protons and the methylene protons [phenyl-(CH_2-Br)$_3$] in the initiator appear at ~7.36 and ~4.46 ppm (not shown), respectively. After the polymerization of 2-ethyl-2-oxazoline, three additional peaks (*e*, *d*, and *c*) at 1.20, 2.32–2.44, and 3.47–3.49 ppm appear corresponding to the polymer backbone. The three aromatic protons shift from 7.36 to 6.85 ppm (peak *b*). The degree of polymerization (*N*) was determined using the ratio of the protons from the polymer backbone at 2.32–2.44 ppm to the three aromatic protons at 6.85 ppm and *N* was close to the theoretical value. In addition, there is no peak corresponding to the original peaks from the initiator, indicating complete consumption of the initiator.

In the second step, the living polymer solution was end-capped with potassium ethyl xanthate, as described previously.[37,38] The polymer was purified by filtration and washed to remove unreacted potassium salt. After end-capping process, two additional peaks appeared at 4.68 ppm (peak *h*) and

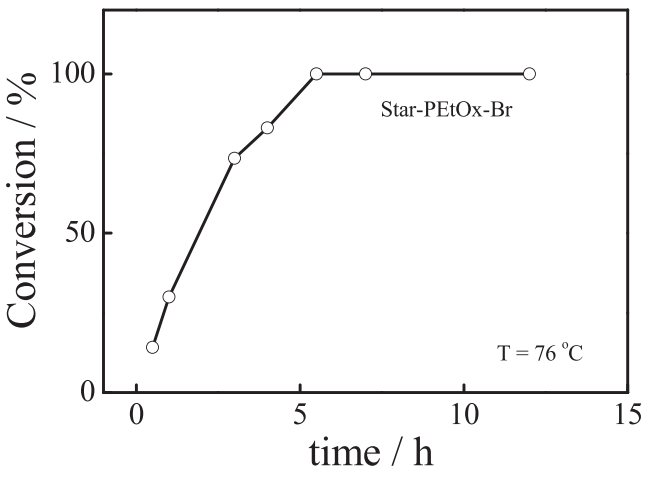


FIGURE 1 Time dependence of the conversion (%) of 2-ethyl-2-oxazoline using 1,3,5-tris (bromomethyl) benzene as the initiator in acetonitrile at 76 °C, where the ratio of the monomer to the initiator (M/I) was 30:1.

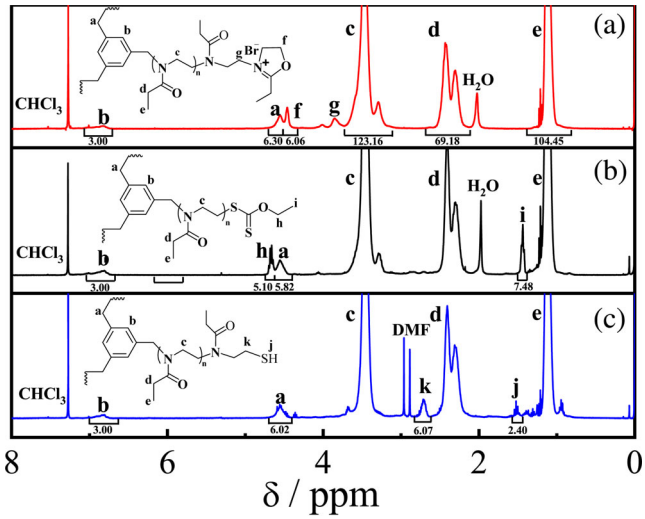


FIGURE 2 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of (a) star-PEtOx-Br, (b) star-PEtOx-xanthate, and (c) star-PEtOx-thiol. [Color figure can be viewed at wileyonlinelibrary.com]

1.43 ppm (peak *i*), respectively, as shown in Figure 2(b). The peak *h* at 4.68 ppm corresponds to the two methylene protons (O—C***H***$_2$—CH_3—) and the peak *i* at 1.43 ppm corresponds to the three methyl protons (O—CH_2—C***H***$_3$) from the xanthate moiety, which confirms the quantitative incorporation of the xanthate end group. The end-group functionality was determined from the peak ratio of the aromatic protons from the initiator at 6.86 ppm (peak *b*) and the xanthate methyl protons at 1.43 ppm, respectively. The calculated functionality was more than 70%.

In the third step, the xanthate group (protected thiol) was cleaved with *n*-butylamine in THF as described previously.[39] During the cleavage process, the thiols were oxidized to disulfides and a gel was formed. Therefore, the gel was suspended in DMF and excessive amount of DTT was added to reduce the polymer to three-arm star PEtOx with trithiol groups. As seen from the ^{1}H NMR spectrum in Figure 2(c), the two peaks from the xanthate end group at 4.68 and 1.43 ppm completely disappeared, indicating that xanthate groups (protected thiol groups) were completely cleaved. Two additional peaks appears at 1.5 and 2.70 ppm corresponding to the thiol proton (—CH_2—CH_2—S***H***) and the methylene protons adjacent to thiol group (—CH_2—C***H***$_2$—SH), which also confirms the successful reduction of the oxidized polymer.

^{13}C NMR was used to further confirm the structure of the star-PEtOx-Br, as shown in Figure S3. After polymerization, four different peaks related to the four different carbons of the polymer were observed, which is in accordance with a previous study.[40] The signals at 173 and 174, 43–46, 25, and 9.33 ppm correspond to the carbonyl carbon (—CH_2—***C***═O—), the methylene carbons (—N—***C***H$_2$—***C***H$_2$—) of the backbone, the pendant methylene group (—***C***H$_2$—CH_3), and the methyl carbon (***C***H$_3$—CH_2—C—), respectively. It should be noted that there is an additional peak at 8.9 ppm, which is due to the

methyl carbon of the oxazolinium ring, as the polymer is not terminated. After termination with potassium ethyl xanthate, this peak disappears and an additional peak appears at 13 ppm, which corresponds to the methyl carbon of the xanthate group. The results from ^{13}C NMR show successful end-group modification, which is in agreement with the ^{1}H NMR data. After cleavage and reduction with DTT, this peak at 13 ppm disappears completely, which shows 100% cleavage of the xanthate end group. The signals of the solvent $CDCl_3$ appear at 77.20 ppm.[38,40]

The end-group xanthate was also confirmed by using UV–visible spectroscopy as xanthate moiety gives absorbance at 282 nm, while the star-PEtOx-Br and star-PEtOx-thiol has no such absorbance, as shown in Figure 3.[41] The UV–visible spectra also show that after cleavage with the base, the peak at 282 nm completely disappears, indicating successful conversion to the star-PEtOx-thiol. To avoid the process of gelation by the oxidation of the star-PEtOx-trithiol, a linear-PEtOx-xanthate was also prepared so that the growing hyperbranched polymer can be terminated. For this purpose, benzyl bromide was used as an initiator to synthesize the linear-PEtOx-xanthate (Fig. S4).

Molecular weights and distribution of all the PEtOx polymers were determined by SEC in THF with narrow distributed polystyrene standards, as shown in Figure 4. The M_n from SEC and ^{1}H NMR were summarized in the Table S1. The SEC curves show narrow distributed and symmetrical chromatograms, indicating that both the mono and trifunctional initiators are efficient and represents fast initiation and slow propagation system.

Hyperbranched PEtOx was successfully synthesized using the mixture of star-PEtOx-xanthate and linear-PEtOx-xanthate using *in situ* cleavage and subsequent oxidation method.

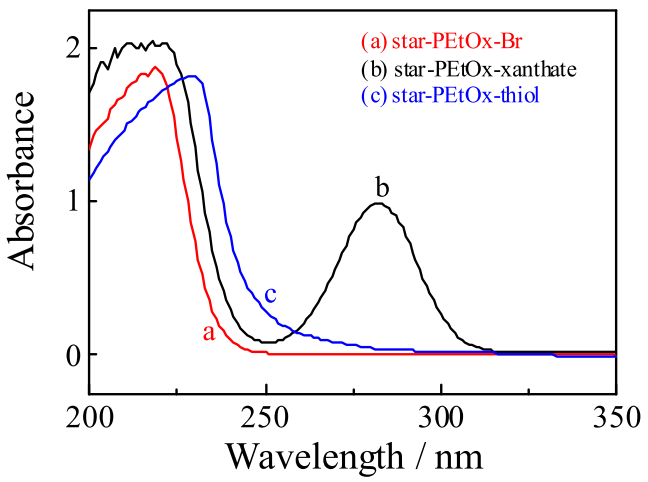


FIGURE 3 UV–vis absorbance spectra of (a) star-PEtOx-Br, (b) star-PEtOx-xanthate, and (c) star-PEtOx-thiol, where the concentrations were 0.5 mg/mL in water. [Color figure can be viewed at wileyonlinelibrary.com]

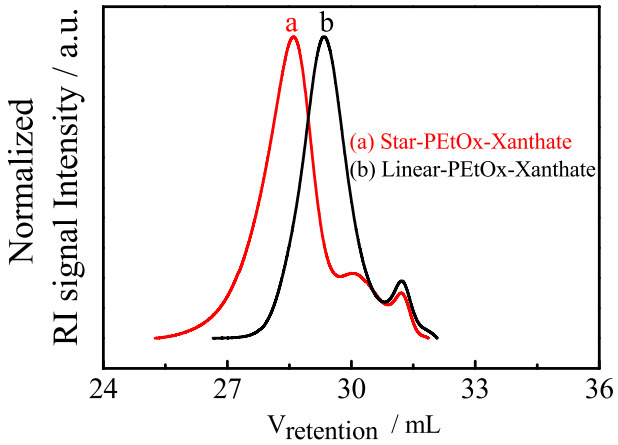


FIGURE 4 SEC traces of (a) star-PEtOx-xanthate and (b) linear-PEtOx-xanthate. [Color figure can be viewed at wileyonlinelibrary.com]

PEtOx gel was formed if only star-PEtOx-xanthate was used, as shown in Figure 5(a). However, our aim was to synthesize hyperbranched PEtOx. Therefore, linear-PEtOx-xanthate was added to avoid gelation. In this study, different weight ratios of the star-PEtOx-xanthate to linear-PEtOx-xanthate were used. Figure 5(c) clearly shows that with the addition of 20% of linear-PEtOx-xanthate hyperbranched PEtOx was formed without the formation of the gel.

The thiol content in the macromonomers and hyperbranched polymers was determined using the Ellman's assay.[32] As shown in Figure 6, after oxidation, the hyperbranched polymer does not show any absorbance at 412 nm, which shows that thiols are completely oxidized. Hyperbranched PEtOx without free thiol groups guarantee the stability of the polymer for further applications like fractionation and hydrolysis to polyethyleneimine. Moreover, the functionality of thiol groups for star-PEtOx-trithiol was ~0.80 by using the absorbance at 412 nm with an extinction coefficient of 13,600 M^{-1} cm^{-1}.

The hyperbranched polymer was formed *in situ* by mixing different ratios of the star and linear PEtOx with xanthate end groups, as shown in Figure 7. These xanthate groups were cleaved by adding *n*-butylamine, which also acts as a base. The thiols were *in situ* oxidized to form hyperbranched polymer. The mechanism is simple that the hyperbranched structure was formed by the star polymer and the gelation was avoided as there was also a monothiol, which acts as an end-capping agent. The polymer mixture with 80% of star polymer and 20% of linear polymer gives the best result, as shown in Figure 7(a,b), that is, the hyperbranched PEtOx with the highest molecular weight but without the formation of the gel in the system. Figure 7 also shows that the molecular weight of the hyperbranched PEtOx increases with the increasing weight ratios of the star to linear PEtOx. Furthermore, the hyperbranched PEtOx can be reduced back to the star-PEtOx-trithiol and linear-PEtOx-thiol using DTT as a reducing agent, as shown in Figure 7(f).

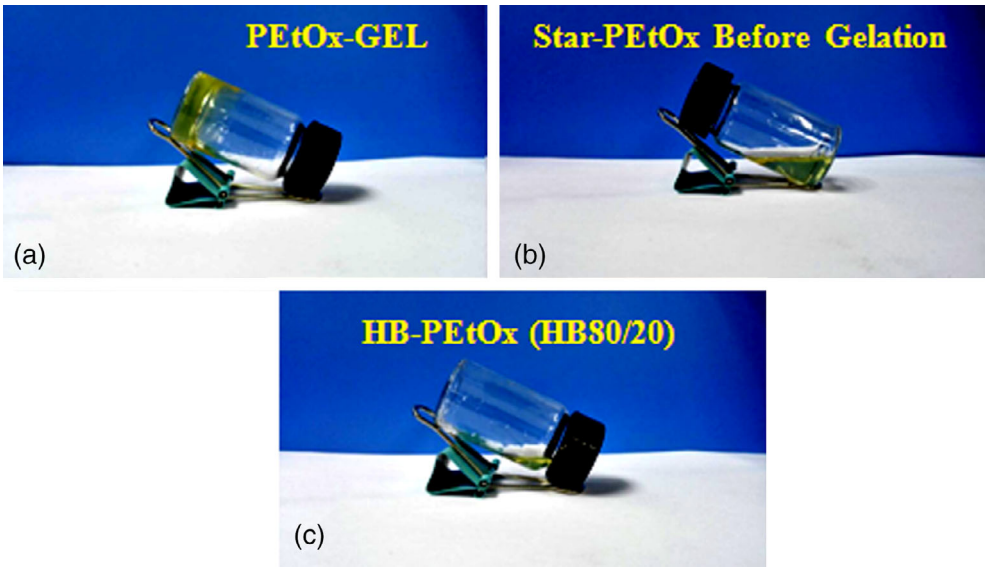


FIGURE 5 Cleavage and oxidation of the star-PEtOx-xanthate and linear-PEtOx-xanthate to form PEtOx gel or hyperbranched PEtOx using different conditions. (a) PEtOx gel formed by oxidation of the star-PEtOx-xanthate (20% w/v) in THF. (b) The star-PEtOx-xanthate (20% w/v) in THF before gelation. (c) HB-PEtOx formed from the mixture of 80% star-PEtOx-xanthate and 20% linear-PEtOx-xanthate (total solution: 20% w/v) [Color figure can be viewed at wileyonlinelibrary.com]

The novel hyperbranched PEtOx was hydrolyzed to a hyperbranched polyethyleneimine using 1 M hydrochloric acid, as described previously.[42] After hydrolysis, the peaks at 2.32–2.44 and 1.20 ppm of the polymer (methylene and pendant methyl protons) completely disappeared, which confirmed the hydrolysis of the star-PEtOx-Br and HB-PEtOx, as shown in Figure S5.

The main advantage of our system is the presence of the disulfide bond, which can be cleaved inside the intracellular environments to small star and linear PEI. Therefore, we determined the cytotoxicity of the star-PEI and HB-PEI using MTT assay. From Figure 8 we can see that IC_{50} of the our hyperbranched PEI (HB-PEI) is 50 μg/mL, indicating that it is

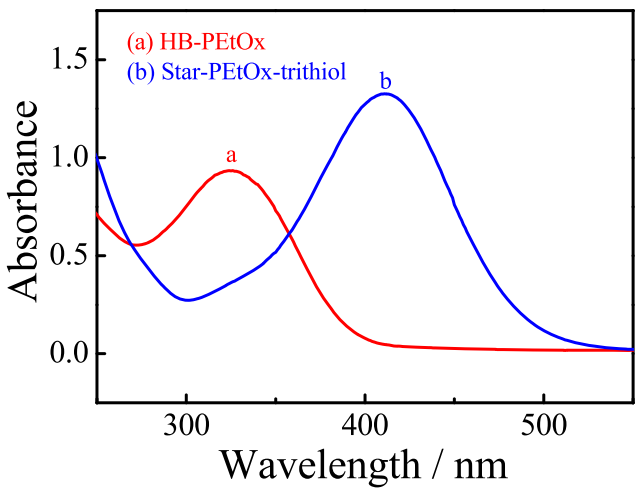


FIGURE 6 UV–vis spectra of (a) hyperbranched PEtOx (b) star-PEtOx-trithiol with the addition of 0.1 mM Ellman's reagent. The polymers concentrations were 5.0 mg/mL and the concentration of the thiol was determined using the Beer–Lambert law. [Color figure can be viewed at wileyonlinelibrary.com]

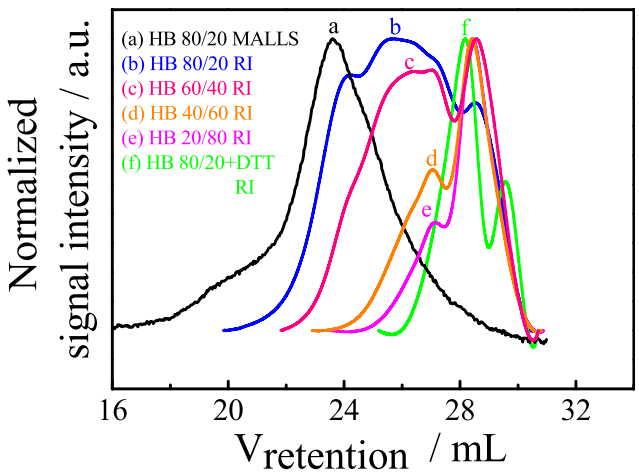


FIGURE 7 SEC traces of (a) HB 80/20 measured by a MALLS detector (HB 80/20 MALLS), where $M_w = 1.2 \times 10^5$ g/mol, (b) HB 80/20 measured by a RI detector (HB 80/20 RI), (c) HB 60/40 RI, (d) HB 40/60 RI, (e) HB 20/80 RI, and (f) hyperbranched polymer HB 80/20 treated with excessive DTT and measured by RI detector (HB 80/20 + DTT RI), where the first and the second numbers represent the weight percentage of the star-PEtOx-xanthate and linear-PEtOx-xanthate. For example, 80/20 means that 1.00 g of star-PEtOx-xanthate (1.00 mmol of xanthate end group) and 0.250 g of linear-PEtOx-xanthate (0.250 mmol of the xanthate end group) were mixed in 6 mL of THF. [Color figure can be viewed at wileyonlinelibrary.com]

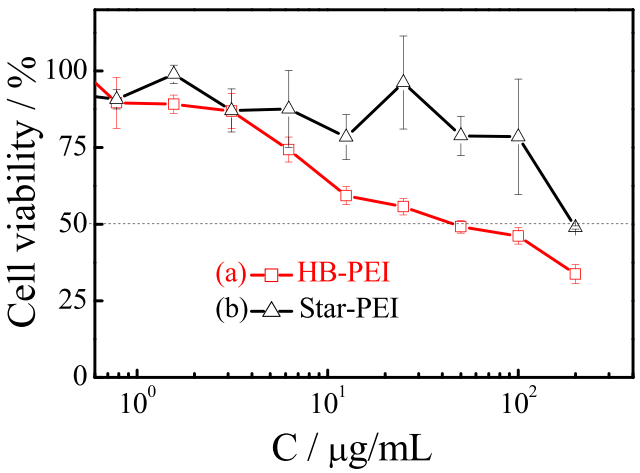


FIGURE 8 Cell viability of HeLa cells as a function of the concentration of polyethyleneimine using MTT assay (a) HB-PEI and (b) star-PEI. [Color figure can be viewed at wileyonlinelibrary.com]

less cytotoxic as compared to the commercial HB-PEI (25 K, IC_{50} = 15 μg/mL), although the molecular weight is as high as $M_w = 5.2 \times 10^4$ g/mol calculated from the molecular weight of the hyperbranched PEtOx sample (HB 80/20), which shows the significance of this system as compared to previously described hyperbranched polyethyleneimines.[43,44] Similarly, the IC_{50} of the star-PEI is 190 μg/mL as the molecular weight of the polymer is less than 2 K g/mol. Previous studies showed that the cytotoxicity of PEI depends upon the molecular weight: the higher the molecular weight, the higher the cytotoxicty will be.[42]

CONCLUSIONS

A novel hyperbranched degradable poly(2-ethyl-2-oxazoline) was synthesized by combining cationic ring-opening polymerization and oxidation of thiol to disulfide groups. Star-PEtOx-Br was synthesized using an efficient trifunctional bromide initiator and end-capped with a protected thiol. A linear-PEtOx-Br was also synthesized and end-capped with xanthate. Hyperbranched PEtOx was then formed by *in situ* cleavage and oxidation process by mixing the two polymers in different ratios. The hyperbranched structure was confirmed from SEC. The hyperbranched PEtOx was hydrolyzed to a novel hyperbranched polyethyleneimine. The result shows that the hyperbranched polyethyleneimine is less cytotoxic than the commercial branched PEI with molecular weight of 25 K.

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