Polymer Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Full pH-range responsive hyperbranched polyethers: synthesis and responsiveness

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Abstract

In order to impart full pH-range responsiveness within biocompatible hyperbranched polyethers, new amphiphilic polyethers, HPMHO-Amines and HPMHO-Carboxys, which had similar molecular structure with hyperbranched PEG, were prepared through ring-opening polymerization and modified by amination or carboxylation. Because of the existence of hydrophobic core and ionization of weak acidic and basic groups on the surface, these hyperbranched polyethers showed reversible pH-response in aqueous solution. And the responsive pH values covered full pH-range and could be readily adjusted by only changing the degree of modification. Cellular experiments showed these pH-responsive hyperbranched polyethers had low cytotoxicity, indicating this novel full pH-range responsive hyperbranched polyether would be a promising functional material for physiological pH relevant bio-application.

Introduction

With its excellent biocompatibility and chemical stability, poly(ethylene glycol)(PEG), as a polyether, and its derivatives have a tremendous variety of applications in food and medicine[1-9]. The PEG is also used to conjugate to various investigational and clinical compounds, such as peptides, proteins and antibodies, to enhance the circulation lifetime of these biologically active molecules (PEGylation)[10-12]. PEG became the first choice in the biologically pharmaceutical industry to improve the pharmacological efficacy[13-15]. However, depending on the inherent structure of linear PEG chain, PEG exhibits a maximum of two functional end groups, limiting its potential for further modification and attaching capacity as a drug carrier[11, 16, 17]. In order to extend the PEG's application, many researchers focus on the preparation of hyperbranched PEG which has plenty of terminal functional groups and can carry multiple drugs, targeting and imaging ligands. Hawker et al.[18] reported a multi-step method to synthesize branched PEG with an aromatic branching unit. Zhu et al.[19] described a one-pot approach to long-chain PEG through

proton-transfer polymerization using PEG and glycidyl methacrylate, and this hyperbranched PEG (HPEG) was biocompatible and could be used as a promising drug carrier. Frey et al.[20] described a random copolymerization of ethylene oxide and glycidol to obtain hyperbranched PEG, which demonstrated a dramatic effect when compared to linear PEG. However, since these hyperbranched polyethers are consisted of hydrophilic segments, hyperbranched PEG is a kind of completely water-soluble hyperbranched polyether, and can't respond to the environmental stimuli. If the stimuli-response is endowed to hyperbranched polyether, the polyether thus obtained would be much more suitable for targeting therapy. In particular, some cellular compartments and tissues have different pH environments compared to normal physiological pH[21-24]. For example, intracellular gene delivery requires a specific pH within a narrow endosomal pH range[25]. However, few pH responsive hyperbranched polyethers were reported. Therefore, the procedure to biocompatible full pH-range responsive hyperbranched polyether is very attractive.

pH-responsive polymers usually are polyelectrolytes which bear weak acidic or basic groups that either accept or release protons in response to changes in environmental pH, such as poly(acrylic acid). And the pH range can be generally modulated by incorporating hydrophobic moieties into the polymer backbone and controlling their amount and distribution. Inspired by this concept, we synthesized a hydrophobic hyperbranched polyether, hyperbranched poly[3-methyl-3-(hydroxymethyl)oxetane] (HPMHO). HPMHO had similar molecular structure with hyperbranched PEG, but due to the hydrophobic branching unit >C< and $-CH_3$ segments, it was water-insoluble. HPMHO was modified and a series of carboxy-modified HPMHO (HPMHO-Carboxys) and amine-modified HPMHO (HPMHO-Amines) were obtained. Because of the existence of hydrophobic core and surface ionizing of acidic or basic groups, these modified HPMHO could respond to pH variation, which contains full of the pH range, from acidic to basic pH. Meanwhile, the responsive pH value of these modified HPMHO could be easily adjusted by controlling the degree of modification. Due to the similar backbone structure with PEG, good biocompatibility was expected.

Experimental

Materials

3-Methyl-3-(hydroxymethyl)oxetane (MHO), boron trifluoride diethyl etherate ($BF_3{\cdot}O(Et)_2$) and CH_2Cl_2 were purchased from Sigma-Aldrich and refluxed with calcium hydride, and purified by reduced or atmospheric pressure distillation. Pyridine was refluxed with anhydrous potassium hydroxide and distilled just before use. Succinic anhydride (SA), N,N'-carbonyldiimidazole (CDI) and ethylenediamine (EDA) (CP grade, SCRC) were used as received. Hyperbranched polyethylenimine (HPEI, water free, Mw = 25 kDa, Mn = 10 kDa) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich and used as received. Dialysis bags (MWCO = 1.0 kDa) were purchased from Shanghai Green Bird Development Co. Ltd., China. Clear polystyrene tissue culture treated 96-well plates were obtained from Corning Costar, and other reagents were obtained from local suppliers and used when received.

Synthesis of HPMHO

Hyperbranched poly[3-methyl-3-(hydroxymethyl)oxetane] (HPMHO) was synthesized by cationic polymerization using $BF_3{\cdot}O(Et)_2$ as an initiator. Under nitrogen atmosphere, CH_2Cl_2 (solution) and $BF_3{\cdot}O(Et)_2$ were put successively into a four-necked flask. Then the CH_2Cl_2/monomer (MHO) solution was dropped into the reactor (monomer/initiator = 2:1). The reaction was kept at 20 ^oC and after 48h stirring, the reaction was quenched. The products were dissolved with ethanol after concentration by rotary evaporation, then precipitated in distilled water and dried at 60 ^oC in vacuum for 4 days in order to obtain final HPMHO.

Synthesis of HPMHO-Amines and HPMHO-Carboxys

Amine-modified hyperbranched poly[3-methyl-3-(hydroxymethyl)oxetane]s (HPMHO-Amines) with different contents of amine terminals were synthesized via two-step modification with N,N'-carbonyldiimidazole (CDI) and ethylenediamine (EDA) successively. HPMHO and CDI with different molar rations were introduced into a flask with DMSO as a solvent. The solution was stirred with a magnetic bar at room temperature for 10 h. Then the DMSO solution of EDA was added into the flask and the solution was stirred at 60 ^oC for 10 h. Most of DMSO was removed by reduced pressure distillation, the residual solution was enclosed in dialysis bag (MWCO = 1.0 kDa), and purified by dialyzing in 2 L deionized water for 72 h. The deionzed water was exchanged for several times. The polymer/water mixture was dried by lyophilization and the product was dried in vacuum oven for 24 h. The products were light yellow powder. The degree of amination of each HPMHO-Amine was measured in terms of its ^1H NMR result. Four HPMHO-Amine samples with amination degrees of 0.25, 0.5, 0.75 and 0.9 were prepared, which named as $Amine_{0.25}$-HPMHO, $Amine_{0.5}$-HPMHO, $Amine_{0.75}$-HPMHO and $Amine_{0.9}$-HPMHO, respectively.

Carboxy-modified hyperbranched poly[3-methyl-3-(hydroxymethyl)oxetane]s (HPMHO-Carboxys) were synthesized from HPMHO and succinic anhydride (SA) by one-step esterification. HPMHO and SA were dissolved in pyridine, respectively. Then the solutions were mixed and stirred with a magnetic bar at 60 ^oC for 24 h. After pyridine was removed with rotary evaporator, the product was dissolved in dimethylsulfoxide (DMSO), enclosed in dialysis bag (MWCO = 1.0 kDa), and purified by dialyzing in 2 L deionized water for 96 h. The polymer/water mixture was dried by lyophilization. Finally, transparent and viscous hyperbranched polyethers with carboxyl groups (HPMHO-Carboxys) were obtained. The degree of carboxylation of each was measured in terms of its ^1H NMR result. Three HPMHO-Carboxy samples with carboxylation degree of 0.5, 0.75 and 0.9 were prepared, which were named as $HPMHO\text{-}Carboxy_{0.5}$, $HPMHO\text{-}Carboxy_{0.75}$ and $HPMHO\text{-}Carboxy_{0.9}$.

^1H NMR (400 MHz, DMSO-d_6, 298 K) δ ppm: 0.74-0.91 (m, -CH_3), 2.45 (s, -OCOCH_2CH_2COO-), 2.72 (s, -OCONHCH_2CH_2NH_2), 3.15 (s, -CH_2OCH_2-), 3.23 (s, -CH_2OH), 3.79-3.83 (d, -NHCOOCH_2-), 3.86-3.90 (d, -CH_2OCOCH_2-), 4.12 (br, -O*H*), 5.37 (br, -CH_2NH_2), 7.26 (br, -CH_2N*H*COOCH_2-), 12.22 (br, -COO*H*).

^{13}C NMR (400 MHz, DMSO-d_6, 298 K) δ ppm: 17.36, 17.63, 17.91 (-*C*H_3), 29.32, 29.38 (-*C*H_2CO-), 40.83 (-OCONH*C*H_2*C*H_2NH_2), 41.41-42.25 (>*C*<), 64.10-64.89 (-*C*H_2OH), 66.31-66.72 (-*C*H_2OCO-), 74.88-74.95 (-CH_2O*C*H_2-), 156.91 (-O*C*ONHCH_2-), 172.46

(-CH_2OCOCH_2-), 174.05 (-CH_2COOH).

IR (cm^{-1}): 3380 ($\nu_{as\ OH}$), 3340 ($\nu_{s\ NH}$), 2965 ($\nu_{as\ CH3}$), 2930 ($\nu_{as\ CH2}$), 2881 ($\nu_{s\ CH3}$), 2858 ($\nu_{s\ CH2}$), 1738 ($\nu_{C\text{-}C=O}$), 1700 ($\nu_{N\text{-}C=O}$), 1536 (δ_{NH}), 1259 ($\nu_{s\ C\text{-}N}$), 1255 ($\nu_{as\ C\text{-}O\text{-}C}$), 1044 ($\nu_{s\ C\text{-}O\text{-}C}$).

pH-induced phase separation behavior of HPMHO-Amines and HPMHO-Carboxys

HPMHO-Amines were dissolved in dilute hydrochloric acid (HCl) aqueous solution to form transparent solution (2 mg/mL). Then sodium hydroxide (NaOH) aqueous solution ($[OH^-]$ = 1 mol/L) was added by dropwise. With the increase of pH value As pH value increasing, the solution turned turbid, and the phase separation occurred. With UV-vis spectrophotometer and pH monitor meter, the light transmittance at 600 nm of HPMHO-Amine was recorded as the pH value increasing. Dynamic light scattering (DLS) measurements were also introduced to track the changing of hydrodynamic diameter (D_h). The pH-responsive phase separation behavior of HPMHO-Carboxy was examined with the similar methods. HPMHO-Carboxys were dissolved in dilute NaOH solution, and HCl solution ($[H^+]$ = 1 mol/mL) was added by dropwise.

The relative cytotoxicity of HPMHO-Amines and HPMHO-Carboxys

The relative cytotoxicity of HPMHO-Amines and HPMHO-Carboxys were estimated with MTT viability assay. COS-7 cells (a cell line derived from kidney cells of the African green monkey) were seeded in 96-well plates at an initial density of 1×10^4 cells/well in 200 μL cell culture medium, and incubated for 24 h to reach 80% confluency. 50 μL culture medium containing various amount of polymer was injected into the growth medium. After the cells were incubated for another 24 h, 20 μL of 5 mg/mL MTT assays stock solution in phosphate buffered saline (PBS) was added into each well. 4 h later, the medium containing unreacted MTT was removed gingerly. Blue formazan crystals were obtained and dissolved in 200 μL DMSO in each well. The absorbance was measured with a Perkin Elmer 1420 Multilabel counter at a wavelength of 490 nm.

Characterization

^1H NMR and ^{13}C NMR of HPMHO-Amines and HPMHO-Carboxys were performed on an Avance 400 M spectrometer (Bruker BioSpin Group, Switz.) using dimethylsulfoxide-d_6 (DMSO-d_6) as solvent at 20 ^oC. Polymers' Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 (Thermo Fisher, USA) with KBr method. The molecular weight was measured by gel permeation chromatography (GPC) on a Perkin-Elmer series 200 system. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano S apparatus equipped with a 4.0 mW laser operating at λ = 633 nm at 20 ^oC and at a scattering angle of 173^o.

Results and discussion

Synthesis and characterization of HPMHO-Amines and HPMHO-Carboxys

Hyperbranched poly[3-methyl-3-(hydroxymethyl)oxetane] (HPMHO) was synthesized by self-condensing ring-opening polymeriazation of 3-methyl-3-(hydroxymethyl)oxtane (MHO), using $BF_3{\cdot}O(C_2H_5)_2$ (monomer/catalyst = 2:1) as an initiator. The structure and property of HPMHO had been well analyzed before[26, 27]. Fig. 1 gives ^{1}H NMR spectrum of HPMHO, showing four groups of distinct peasks: -C*H*$_3$ at 0.74-0.87 ppm, -C*H*$_2$OC*H*$_2$- at 3.15 ppm, -C*H*$_2$OH at 3.23 ppm and –O*H* at 4.12 ppm. Similarly, four groups of distinct peaks appear in HPMHO's ^{13}C NMR as shown in Fig. 2: -*C*H$_3$ at 17-18 ppm, >*C*< at 41-42 ppm, -*C*H$_2$OH at 64-65 ppm and –*C*H$_2$O*C*H$_2$- at 74-75 ppm. The degree of branching (DB) of HPMHO is calculated according to the quantitative NMR measurement and its value is 0.41. Molecular weight is 6300 and its distribution of HPMHO is 1.85 from GPC testing. Although HPMHO is of plenty of terminal hydroxyls in its chemical structure, but due to the hydrophobicity of methyl and methylene molecular groups, the obtained HPMHO isn't soluble in H_2O.

A variety of amine-modified and carboxy-modified HPMHO derivatives were synthesized by two-step amination and one-step esterification, respectively. Four HPMHO-Amines and three HPMHO-Carboxys with different degree of modification were prepared. HPMHO-Amines were light yellow powders with good solubility in water. Fig. 1 shows four new signals after modification at 2.72, 3.79-3.83, 5.37 and 7.26 ppm appearing in the ^{1}H NMR spectrum of HPMHO-Amine, which can be assigned to the protons of methylene in ethylenediamine unit (-OCONHC*H*$_2$C*H*$_2$NH$_2$), methylene (-NHCOOC*H*$_2$-), amine groups (-CH$_2$N*H*$_2$) and urethane groups (-CH$_2$N*H*COOCH$_2$-). The intensity of hydroxyl at 4.12 ppm (-O*H*) disappears and methylene at 3.23 ppm (-C*H*$_2$OH) decreases a lot. Based on the change of peak areas at 3.1-3.3 ppm (-C*H*$_2$OC*H*$_2$- and –C*H*$_2$OH) and 3.79-3.83 ppm (-NHCOOC*H*$_2$-), the degrees of amination can be calculated, and four samples are named HPMHO-Amine$_{0.25}$, HPMHO-Amine$_{0.5}$, HPMHO-Amine$_{0.75}$ and HPMHO-Amine$_{0.9}$, according to degrees of each amination. ^{13}C NMR spectrum in Fig. 2 confirms these conclusions. Some new peaks appear in its ^{13}C NMR spectrum. The signals at 40.83, 66.31-66.72 and 156.91 ppm are assigned to methylene in the ethylenediamine units, methylene near to urethano (-*C*H$_2$OCONH-) and carbonyl units in urethane (-CH$_2$O*C*ONH-). In the meanwhile, the intensity of methylene (-*C*H$_2$OH) at 64.7 ppm decreases dramatically.

HPMHO-Carboxys are sticky solid, and soluble in water to form transparent solution. In its ^{1}H NMR and ^{13}C NMR spectra (Fig. 1 and 2) several peaks appear after the carboxylation and the peaks assigned to methylene and hydroxyl in -CH$_2$OH decreases or disappears. The degrees of carboxylation were calculated via similar method with HPMHO-Amines, and three samples are named as HPMHO-Carboxyl$_{0.5}$, HPMHO-Carboxy$_{0.75}$ and HPMHO-Carboxy$_{0.9}$ according to each's respective degrees of carboxylation. Furthermore, the number-average molecular weights of HPMHO-Amines and HPMHO-Carboxy were calculated. And the corresponding data are listed in Table 1.

Table 1 Reaction conditions and characterization data.

Sample	Feed ratio (molar ratio)	Degree of amination (or carboxylation) [a]	Mn ($\times 10^4$) [b]
HPMHO	0	0	0.63
HPMHO-Amine$_{0.25}$	0.25	0.253	0.76
HPMHO-Amine$_{0.5}$	0.5	0.511	0.90
HPMHO-Amine$_{0.75}$	0.75	0.749	1.02
HPMHO-Amine$_{0.9}$	1.2	0.905	1.11
HPMHO-Carboxy$_{0.5}$	0.5	0.501	0.94
HPMHO-Carboxy$_{0.75}$	0.75	0.748	1.09
HPMHO-Carboxy$_{0.9}$	1.2	0.901	1.19

[a] Degree of amination (or carboxylation) was calculated according to ^{1}H NMR results.

[b] Mn of HPMHO was measured by GPC, and other polymer's were calculated based on NMR results.

pH-induced phase transition behavior of HPMHO-Amines and HPMHO-Carboxys

HPMHO has a dendritic molecular structure which is of plenty of hydroxyls. However it is hydrophobic due to the hydrophobicity of methyl and methylene molecular groups in its chemical structure, and is insoluble in water at any temperature. Both amination and carboxylation endow HPMHO with not only hydrophilicity but also a reversible pH-induced phase transition. HPMHO-Amines and HPMHO-Carboxys are highly soluble and stable in acidic environment and alkaline environment, respectively. Once the pH is changed, the aqueous solubility of polymers will transform. For instance, a transparent aqueous solution of HPMHO-Amine becomes opaque at a specific pH with addition of sodium hydroxide (NaOH). Decreasing pH value by adding hydrochloric acid (HCl), the solution becomes transparent again. Furthermore, this reversible phase transition occurs repeatedly for many times. All of these phenomena indicate that the obtained HPMHO-Amines are pH-responsive and their aqueous solutions possess the pH-induced phase transition behavior.

Similar phenomena are observed in HPMHO-Carboxys aqueous solutions, which have an opposite trend when the solutions' pH value change. This pH-induced phase transition is caused mainly by two factors, namely, the hydrophobic core and ionizing of surface amine or carboxy. The ionizing of organic acid or alkali is incomplete, with the equilibrium of ionizing. For amine, acidic aqueous environment promotes its ionization, so the aqueous solubility of HPMHO-Amines is improved dramatically. As pH value increases, more and more amino cations in HPMHO-Amines are deprotonated, resulting in the hydrophobicity enhancement and subsequent phase transition.

These reversible pH-induced phase transition behaviors of HPMHO-Amines and HPMHO-Carboxy are recorded by turbidimetry measurement on a UV-vis spectrometer with a pH meter. The influence of amination or carboxylation on pH-induced phase transition of HPMHO-Amines or HPMHO-Carboxys is presented in Fig. 3. For HPMHO-Amine$_{0.25}$, HPMHO-Amine$_{0.5}$, HPMHO-Amine$_{0.75}$ and HPMHO-Amine$_{0.9}$, the optical transmittance of aqueous solutions starts to decrease when pH values increase to 7.48, 8.28, 8.76 and 9.77, and then the solutions become totally opaque at pH = 8.57, 9.16, 10.1 and 11.5, accordingly. These

results indicate that, as amination increases, the responsive pH value of HPMHO-Amines increases. This regularity confirms our hypothesis, that this pH-induced phase transition is closely related to the amount of surface primary amine in the hyperbranched structure. Consequently, the responsive pH value can be readily adjusted by only controlling amination, which is not available for hydrazone bond and other pH-responsive systems.

For HPMHO-Carboxys, we also can manipulate the pH-induced phase transition behaviors by adjusting the degree of carboxylation. For HPMHO-Carboxy$_{0.5}$, HPMHO-Carboxy$_{0.75}$ and HPMHO-Carboxy$_{0.9}$, the optical transmittance of aqueous solutions starts to decrease when pH values decrease to 6.95, 5.41 and 3.47, and then the solutions become totally opaque at pH = 4.04, 3.46 and 1.76, accordingly. Thus, a full pH-range responsive polymer system was obtained, and the responsive pH value can be adjusted by changing the degree of modification and with different modification methods, such as amination and carboxylation.

Laser light scattering (DLS) was used to provide a further understanding of the pH-induced phase transition behavior of HPMHO-Amines and HPMHO-Carboxys. The changes in the hydrodynamic size (D_h) of HPMHO-Amine$_{0.9}$ and HPMHO-Carboxy$_{0.9}$ were measured by DLS in their aqueous solution (2 mg/mL) at various pH value, and the results are shown in Fig. 4 and Fig. 5. DLS data show that micelles are formed at all pH values for both HPMHO-Amines and HPMHO-Carboxys, from acidic to basic conditions, and their size varies with the pH values. For HPMHO-Amine$_{0.9}$, no obvious changes in the D_h occur at pH values up to 9.62 (D_h remains about 70 nm). As HPMHO-Amine is consisted of hydrophobic polymeric core and weak alkali surface of amine groups, it is expected to be amphiphilic and supposed to be dispersed as nanomicelles with hydrophobic segment as its core and hydrophilic segment as its shell. In acidic condition most of amine groups are protonated, preventing the aggregation, so the micelles will disperse in the aqueous solution very well. In neutral or subalkaline environment, the primary amine group (-NH_2) in HPMHO-Amines becomes less charged or deprotonated. This makes HPMHO-Amines less hydrophilic and the hydrophobicity of micelles is enhanced, which promotes micelles aggregation. As shown in Fig. 4B, the hydrodynamic size of HPMHO-Amine$_{0.9}$ becomes larger as the alkaline of solution enhances, and a sharp increase to about 200 nm takes place as pH increases up to 10.3. As the pH continues increasing, the D_h will be over 1 μm.

For HPMHO-Carboxy aqueous systems, based on similar mechanism, HPMHO-Carboxy will form nanomicelles in its aqueous solution. In the neutral or acidic environment, its hydrophobicity is enhanced, and the polymeric micelles aggregate. D_h increases significantly when pH decreases to 3.17, and finally increases to 1200 nm when pH is 1.70. DLS experiments confirm the results of UV-vis measurements.

In vitro cytotoxicity

Since HPMHO-Amines and HPMHO-Carboxys have stimuli-response, they can be used as promising materials for biomedical applications. To evaluate the potential for biomedicine, the cytotoxicity of HPMHO-Amines and HPMHO-Carboxys against COS-7 cells are studied and compared with HPEI using MTT assay. It has been well reported that hyperbranched polyether, such as polyglycerol (HPG), is highly biocompatible [28, 29]. Since HPMHO is a polyether and has a similar chemical structure with HPG, the good biocompatibility of HPMHO derivatives is expected. The cytotoxicity to COS-7 cells (a cell line derived from kidney cells of African green

monkey) was evaluated by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Fig. 6 shows the cell viability after 24 h incubation with HPMHO-Amine$_{0.25}$, HPMHO-Amine$_{0.5}$, HPMHO-Amine$_{0.75}$, HPMHO-Amine$_{0.9}$, HPEI, HPMHO-Carboxy$_{0.5}$, HPMHO-Carboxy$_{0.75}$ and HPMHO-Carboxy$_{0.9}$ at various concentrations. For HPMHO-Carboxys, when the concentrations are up to 1 mg/mL, the viability of COS-7 cells is still higher than 62%, and the cytotoxicity of HPMHO-Carboxys shows no significant difference, indicating that they possess good biocompatibility and might be used as drug delivery carriers.

HPMHO-Amine has amine groups and in its neutral aqueous solution it exits in the state of polycation, which is well-known for its cytotoxicity, such as HPEI. Fig. 6B gives the cell viability after incubation with HPMHO-Amines, compared with HPEI. For HPMHO-Amines, no significant cytotoxicity is observed at the concentration up to 0.01 mg/mL after 24 h incubation, and at 0.1 mg/mL, still more than 40% of the cells survives. In the contrast, HPEI shows cytotoxicity when the concentration is 0.01 mg/mL, and at 0.1 mg/mL, only about 15% of the cells survives. It was reported that the positive charge of amine groups would increase the cytotoxicity[30]. Therefore, the relative low cytotoxicity of HPMHO-Amines might be of that only its terminal groups are positively charge in its aqueous solution, but its hyperbranched polyether core doesn't. So HPMHO-Amines have relatively low cytotoxicity, compared with HPEI, and so exhibit their potential for biomedical application, such as gene delivery.

Conclusions

A series of pH responsive polymers, HPMHO-Amine and HPMHO-Carboxy, were synthesized successfully through self-condensing ring-opening polymerization of 3-methy-3-(hydroxymethyl)oxetane, and followed by amination with ethylenediamine and carboxylation with succinic anhydride, respectively. The structures and pH-induced phase separation behaviors of HPMHO-Amines and HPMHO-Carboxys were investigated by NMR, FTIR, GPC, UV-vis and DLS. Significantly, the responsive pH value could be adjusted, and so regulated to the target value, by changing the feed ratio during the modification. DLS results showed that the polymeric micelles would aggregate when the pH reaches to the critical pH value. Cellular experiments indicated that HPMHO-Carboxys had low cytotoxicity and excellent biocompatibility, and so were promising stimuli-responsive drug carriers; HPMHO-Amines had lower cytotoxicity than HPEI, and exhibited their potential as a stimuli-responsive polycation in gene delivery. These results would expand the biomedical applications of hyperbranched polyether and its derivatives extensively

Acknowledgement

I gratefully acknowledge the (valuable help and great) support from Innovation Program of Shanghai Municipal Education No. 12ZZ062, the Fundamental Research Funds for the Central Universities, the Shanghai Natural Science Foundation and Programme of Introducing Talents of Discipline to Universities No. 111-2-04.

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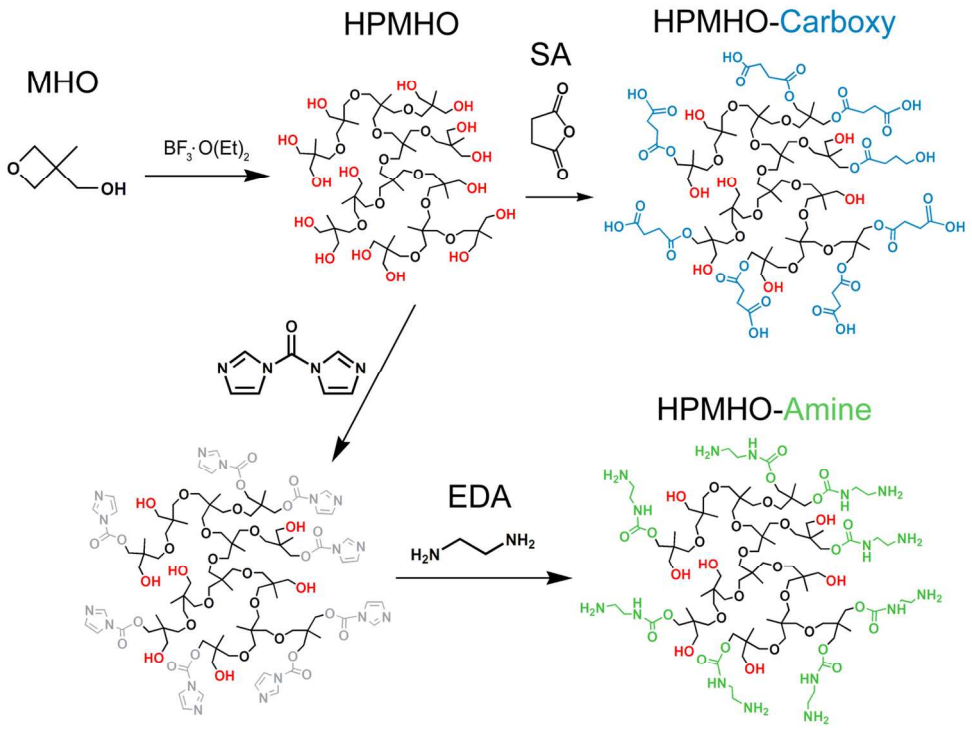

Scheme 1. Synthesis of HPMHO-Amines and HPMHO-Carboxys.
135x104mm (300 x 300 DPI)

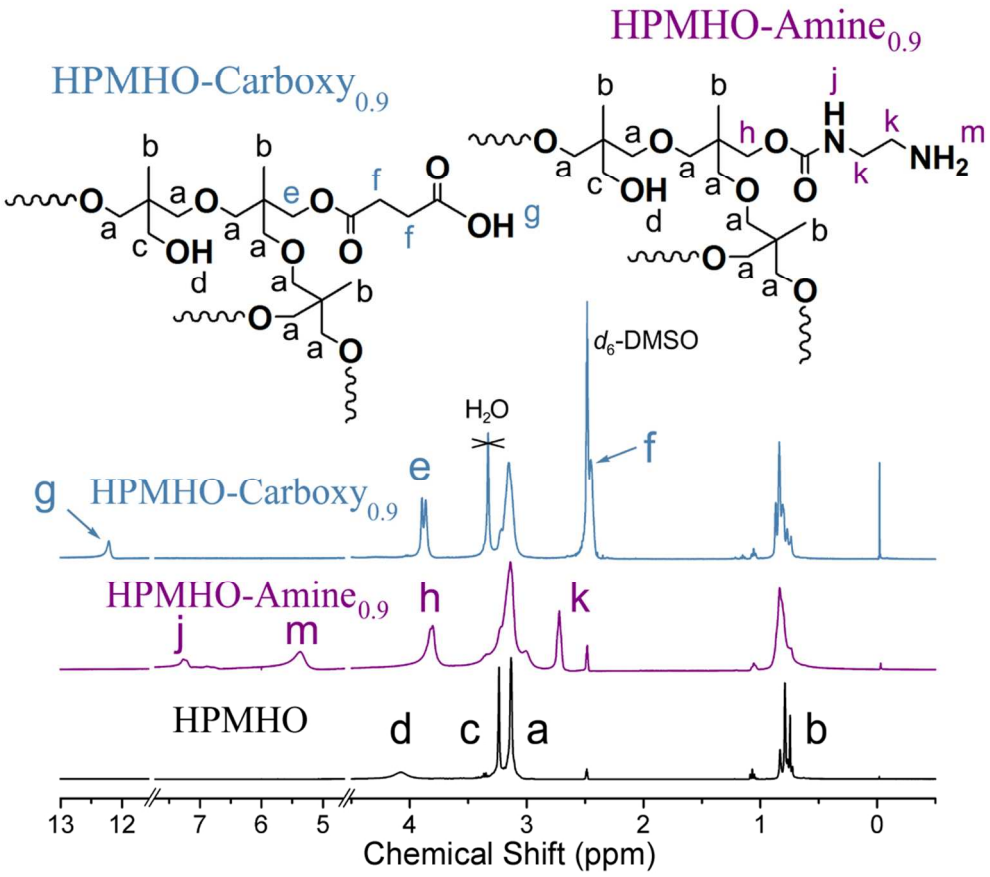

Figure 1. ^{1}H NMR spectra of HPMHO, HPMHO-Amine and HPMHO-Carboxy and their chemical structure.
101x92mm (300 x 300 DPI)

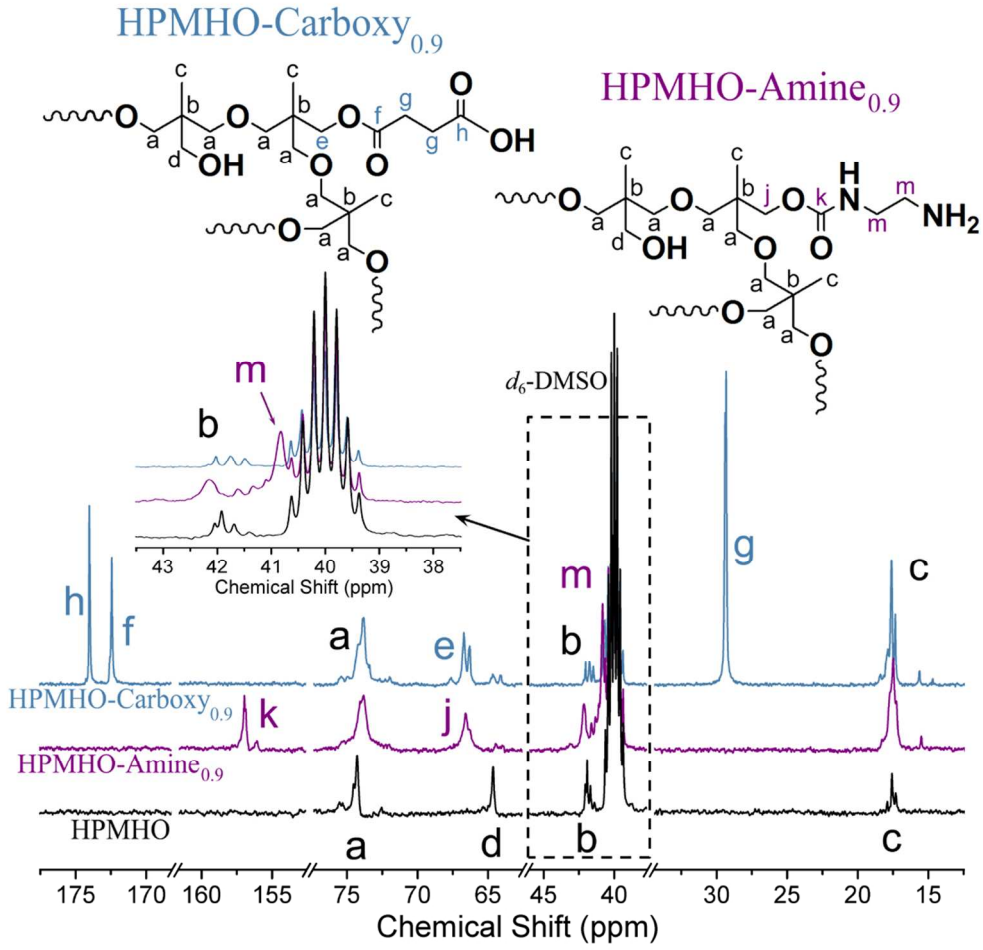

Figure 2. ^{13}C NMR spectra of HPMHO, HPMHO-Amine and HPMHO-Carboxy and their chemical structure.
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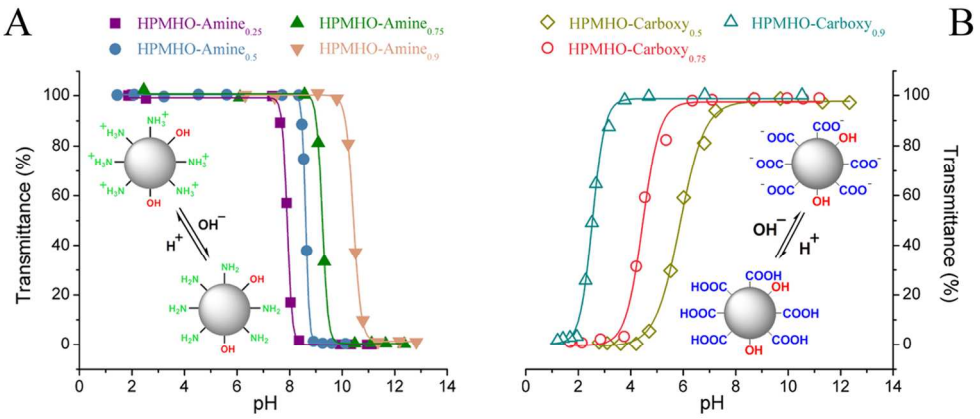

Figure 3. pH-responsive behaviors of (A) HPMHO-Amines and (B) HPMHO-Carboxys aqueous solutions.
122x55mm (300 x 300 DPI)

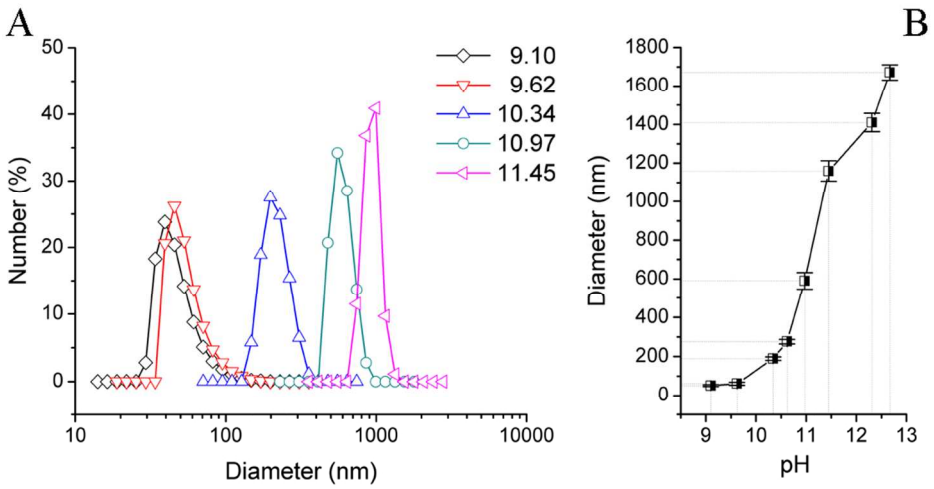

Figure 4. (A) DLS plot of HPMHO-Amine$_{0.9}$ aqueous solution at several pH values; (B) Hydrodynamic size of HPMHO-Amine$_{0.9}$ as a function of pH value.
101x51mm (300 x 300 DPI)

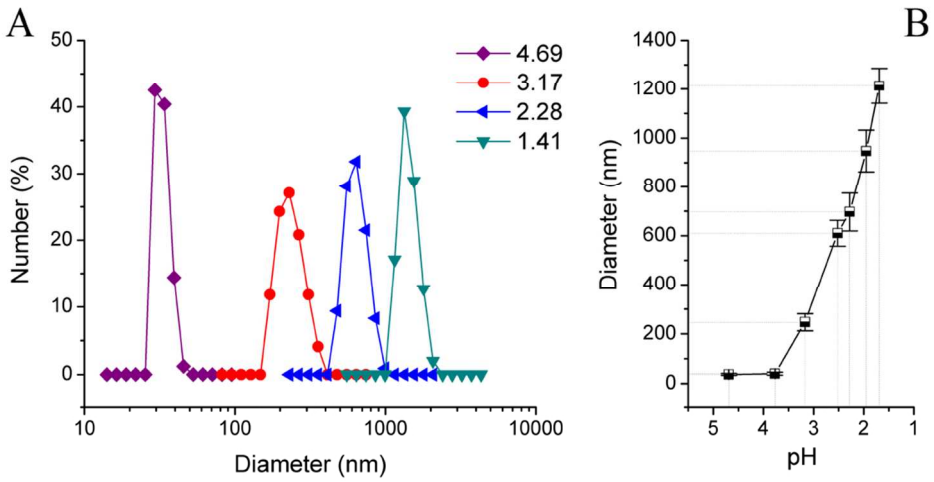

Figure 5. (A) DLS plot of HPMHO-Carboxy$_{0.9}$ aqueous solution at several pH values; (B) Hydrodynamic size of HPMHO-Carboxy$_{0.9}$ as a function of pH value.
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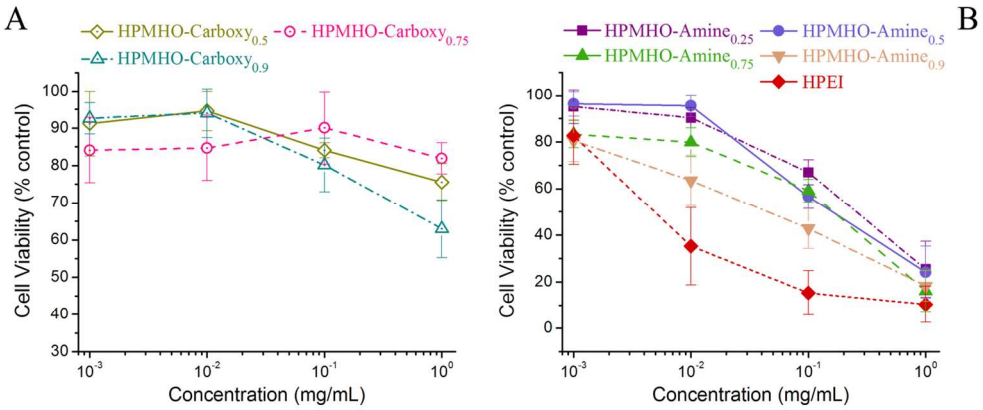
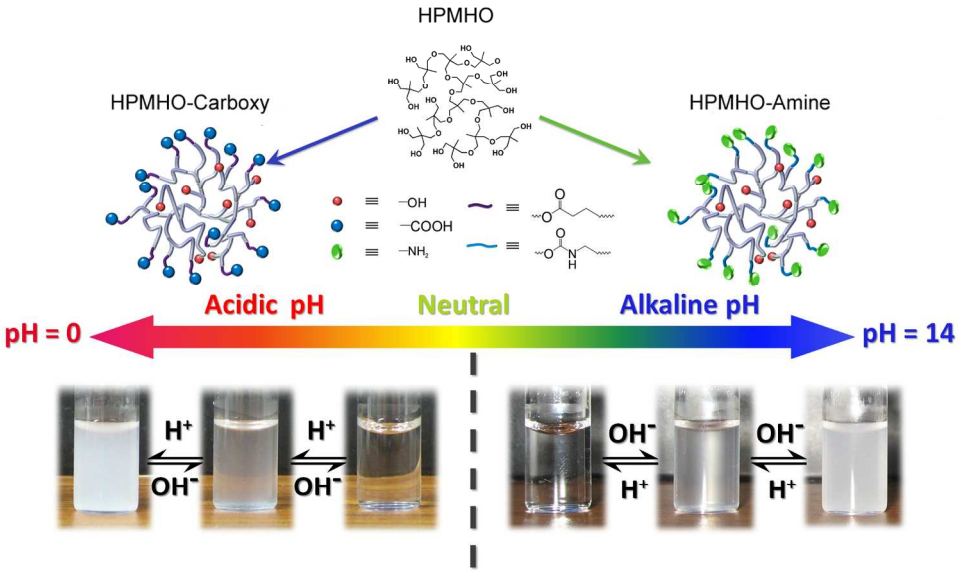


Figure 6. Cell viability of COS-7 cells against HPMHO-Carboxys, HPMHO-Amines and HPEI after cultured with different concentration (mean ± S.D., n = 5).
270x115mm (150 x 150 DPI)



Graphical abstract
281x168mm (180 x 180 DPI)