A New End Group Structure of Poly(ethylene glycol) for Hydrolysis-Resistant Biomaterials

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INTRODUCTION Because of their excellent biocompatibility, poly(ethylene glycol) (PEG) and its derivatives have been widely used in biomedical applications, especially in drug PEGylation, biomaterial surface modification, and hydrogel-based implants and tissue scaffolds.[1–8] To be incorporated into a hydrogel structure, the hydroxyl end group of PEG is usually and conveniently reacted with acryloyl chloride to form an acrylate structure (PEG-A, Scheme 1),[9–11] which subsequently undergoes either a free radical polymerization or the Michael-type addition reaction with thiol-containing molecules.[11–14] Consequently, the formed hydrogel structure contains ester linkages at the crosslink points, and their hydrolysis (half time of the ester bonds in the water-rich environment is on the order of days at pH 7.4 and 37 °C) will result in disintegration of the gel,[15–17] which is an advantage for applications requiring gel degradation but a disadvantage for longer term applications such as vitreous, cartilage, and nucleus pulposus replacement.

For such applications requiring hydrolysis-resistant PEG-based biomaterials, PEG with vinyl sulfone end groups (PEG-VS) seems the only structure that can be obtained through direct reaction with hydroxyl-ended PEG.[18,19] However, the synthesis of PEG-VS either involves PEG reaction with divinyl sulfone, which is highly toxic, or a lengthy multiple-step reaction route. Therefore, alternative PEG end group structures should be developed for hydrolysis-resistant biomaterials.

In this study, we designed and synthesized such a structure in which the PEG chain is linked to the α-carbon atom of a methacrylate group and forms an α-PEG-MA structure (Scheme 1). In this structure, the electron-withdrawing ester group is still connected to the vinyl group, making the latter active for Michael-type addition reaction and possible free radical polymerization. However, unlike in the PEG-A system, ester hydrolysis will no longer affect the linkage of PEG to the gel structure (Scheme 1). As shown below (Scheme 2), the synthesis of the new structure is a one-step reaction with high yield using commercially available reagents. The reactivity of the new structure in the Michael-type addition was found comparable to that of the conventional acrylate end group, and the hydrolysis-resistant property of the formed hydrogel was demonstrated in this study.

EXPERIMENTAL

Materials and Methods

PEG (MW 2000, PEG2K), sodium hydride (NaH), 4-dimethylaminopyridine (DMAP), triethylamine (TEA), L-cysteine hydrochloride, and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Alfa Aesar. Acryloyl chloride was purchased from TCI Chemical. 4Arm-PEG-SH (MW 10,000) was purchased from Jen Kem Technology. Ethyl 2-(hydroxymethyl) acrylate (EHMA) was purchased from Jiachen Chemical (China). Phosphorus tribromide (PBr_3) was purchased from Sinopharm Chemical (China). All reagents were used as received without further purification.

NMR spectra were recorded on a 300-MHz JOEL spectrometer. UV tests were carried out on a PGeneral TU-1810 UV–Vis spectrophotometer. Matrix assisted laser desorption ionization (MALDI)-time-of-flight mass spectrometer (TOF MS) was conducted using an Applied Biosystems 4800 Plus TOF-MS and α-cyano-4-hydroxycinnamic acid (CHCA) was used as matrix.

Synthesis of Ethyl 2-(Bromomethyl) Acrylate (EBrMA)[20]

EBrMA is commercially available; but in this study, we synthesized it in-house following the protocol in literature. EHMA (7.8 g, 60 mmol) was dissolved in 60 mL diethyl ether, and PBr_3 (2.0 mL, 21 mmol) was slowly added while the vessel was cooled with an ice bath. Afterward, the mixture was warmed to room temperature and stirred for 3 h. After adding 5 mL water, the mixture was extracted with hexane for three times. The organic solutions were combined, washed with brine, and dried with $MgSO_4$. The solvent was removed in a rotary evaporator at 60 °C to give the final product (7.2 g, 90%).

Additional Supporting Information may be found in the online version of this article. Correspondence to: Y. Huang (E-mail: yanbin@tsinghua.edu.cn)
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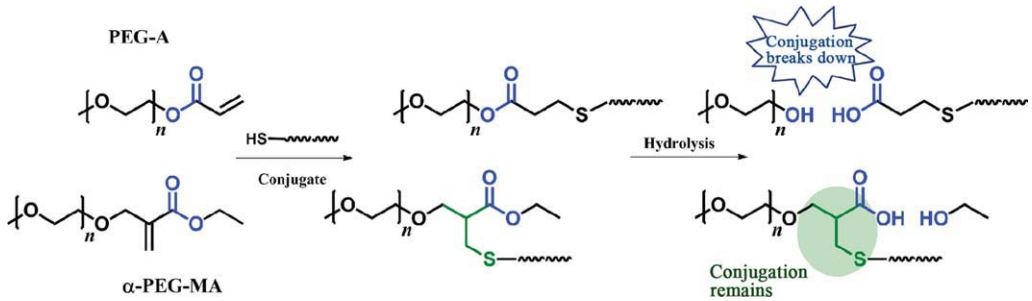


SCHEME 1 Schematic illustration of hydrolysis effect on biomaterials based on traditional acrylate PEG and the new α-PEG-MA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

^{1}H NMR ($CDCl_3$): 1.3 ppm (t, 3H, —CH_3), 4.16 ppm (s, 2H, —CH_2—Br), 4.25 ppm (q, 2H, —CH_2—O—), 5.9 and 6.3 (s, 1H, =CH_2).

Synthesis of PEG-A

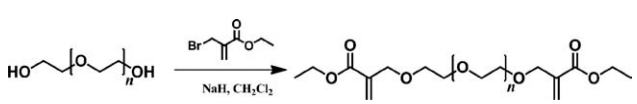
PEG2K (10 g, 5 mmol) was dissolved in 200 mL dry tetrahydrofuran, and added with DMAP (305 mg, 2.5 mmol) and TEA (4.34 mL, 30 mmol). Then acryloyl chloride (2.45 mL, 30 mmol) was added dropwise, and the reaction was carried out overnight at room temperature. The resulting solution was filtered, condensed by rotary evaporation, and precipitated with diethyl ether.

^{1}H NMR (D_2O): 3.6 ppm (200H, PEG backbone), 4.25 ppm (t, 4H, CH_2—O—C (O)—), 5.9 ppm (d, 2H, —C(O)—CH=), 6.1 and 6.4 (q, d, 2H, —CH=CH_2).

Synthesis of α-PEG-MA

PEG2K (10 g, 5 mmol) was dissolved in 200 mL dry dichloromethane, and NaH was added to the solution, at threefold molar excess over OH groups. After that, EBrMA (2.8 mL, 20 mmol) was added. The reaction was carried out at room temperature overnight. The mixture was then neutralized with acetic acid and filtered, and the solvent was removed by rotary evaporation. After redissolving, the residual in tetrahydrofuran and filtration, the solution was condensed and precipitated with diethyl ether. The product was washed with diethyl ether two times and vacuum dried (yield > 90%).

^{1}H NMR (300 MHz, D_2O, Fig. 1): δ (*ppm*) 1.23 (t, 6H, C***H***$_3$—), 3.62 (s, 200H, PEG backbone), 4.17–4.21 (t, s, 8H, —C***H***$_2$—C(O)—O—, —O—C***H***$_2$—C(=CH_2)—), 5.90 (s, 1H, —C=C***H***$_2$), 6.31 (s, 1H, —C=C***H***$_2$). ^{13}C NMR (300 MHz, $CDCl_3$, Supporting Information Fig. S4): δ (*ppm*) 14.21 (***C***H_3—), 60.62 (CH_3—***C***H_2—), 69.24 (—C(=CH—$_2$)—***C***H_2—O—), 70.56 (PEG backbone), 125.50 (—C=***C***H_2), 137.27 (—***C***=CH_2), 165.80 (—***C***=O). FT-IR (KBr, Supporting Information Fig. S7): ν (cm^{-1}) 2887, 1718, 1643, 1470, 1344, 1281, 1424, 1147, 1113, 1063, 960, 843.



SCHEME 2 Synthesis of α-PEG-MA.

MW (MALDI-TOF MS, Fig. 2 and Supporting Information S1): average M_n = 2188, M_w/M_n = 1.01. Melting point (differential scanning calorimetry (DSC), Supporting Information Fig. S6): 43.8 °C; degradation temperature (thermal gravimetric analysis (TGA), Supporting Information Fig. S5): 191.9 °C.

Reactivity Comparison of α-PEG-MA and PEG-A with Thiol

The reactivity of α-PEG-MA and PEG-A with thiol was evaluated using cysteine as the model thiol compound and monitoring thiol concentration with Ellman's assay (DTNB).[17] Briefly, 1 mM solution of cysteine and 0.5 mM PEG derivative (containing 1 mM double bond), prepared in phosphate buffer solution (PBS) buffer (0.2 M, pH 8.0) were mixed at volume ratio of 1:1. At scheduled times, 1 mL of the reaction mixture was mixed with 9 mL DTNB solution (0.1 mM), and the absorbance at 412 nm was measured as an index of thiol concentration.

Gel Preparation and Swelling Experiments

α-PEG-MA (or PEG-A) and 4arm-PEG-SH were first dissolved in PBS (0.2M, pH 7.4) at a concentration of 10% (w/v) separately. The two solutions were then mixed at a 5:2 volume ratio (thiol:vinyl group molar ratio = 1:1) and placed into disc-shaped vessels (1 mL each). After being left overnight to ensure complete reaction, the gels were taken out and immersed in PBS (pH 7.4, 20 mM) at room temperature

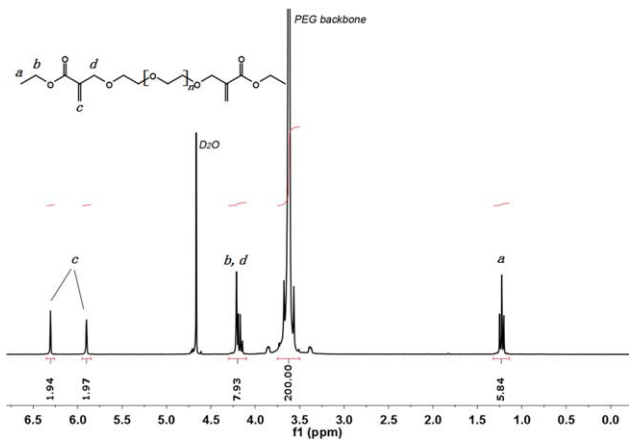


FIGURE 1 ^{1}H NMR spectrum of α-PEG-MA (D_2O, 300 MHz). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

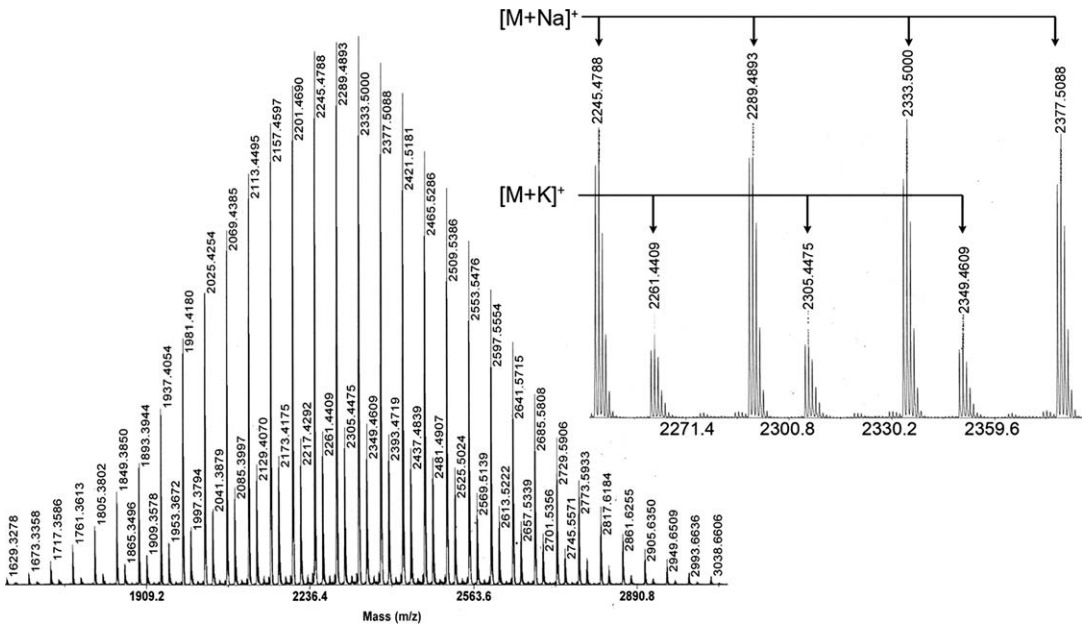


FIGURE 2 MALDI-TOF MS spectrum of α-PEG-MA.

for swelling studies. The swelling ratio, Q, was calculated as: $Q = W_t/W_0$, where W_t is weight at time t and W_0 is the initial weight of the gel.

RESULTS AND DISCUSSION

The α-PEG-MA synthesis was convenient, and the structure was confirmed by ^{1}H NMR (in D_2O) and MALDI-TOF MS analysis. The ^{1}H NMR spectrum was shown in Figure 1. All the resonance peaks were identified and their peak area ratios were found to be consistent with the target structure. The degree of vinyl end group, calculated from the integration area ratio of the proton resonance peak of C=CH_2 to that of PEG backbone was >97%. This high conversion means that the sodium alkoxide, formed by hydroxyl group of PEG reacting with NaH, selectively reacted with Br-group and not added to the C=C double bond, which may result in polymer chain coupling. This high conversion was also verified by the MALDI-TOF MS spectrum (Fig. 2 and Supporting Information Fig. S2), which showed only the targeted α-PEG-MA peaks, ionized with sodium and potassium.

The reactivity of this new PEG end group with thiol was evaluated and compared with that of the usually used PEG-A structure. As shown in Figure 3, the reaction of cysteine with both α-PEG-MA and PEG-A was complete within 45 min at pH 8.0, and the two PEG structures showed comparable reactivity.

To verify the hydrolysis stability of the hydrogel formed by the new PEG structure and 4arm-PEG-SH through the Michael-type addition reaction, swelling studies were carried out and compared with those of PEG-A and 4arm-PEG-SH. The swelling

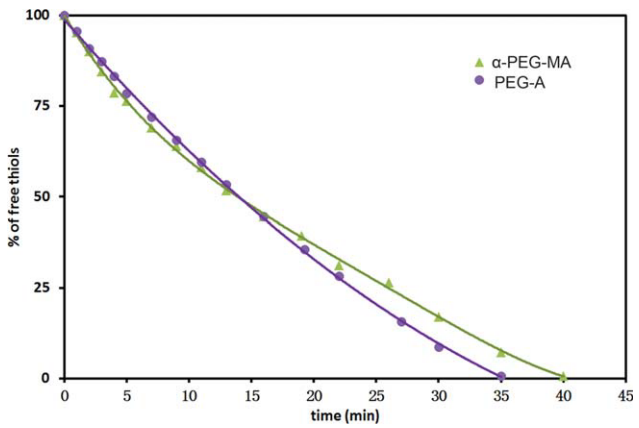


FIGURE 3 Reaction of cysteine with α-PEG-MA (▲) and PEG-A (●), carried out in PBS at pH 8.0 and room temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

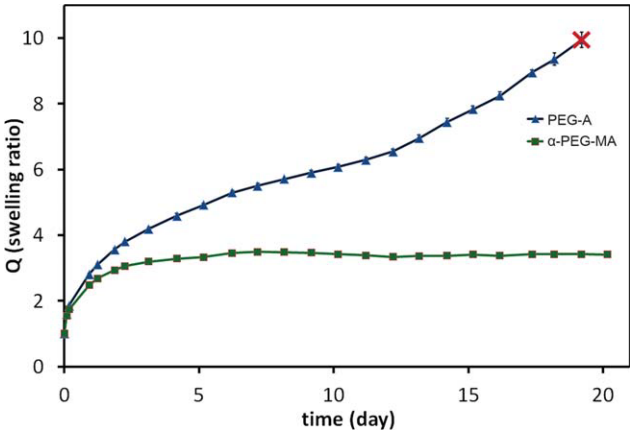


FIGURE 4 Swelling ratio of gels prepared by α-PEG-MA (■) and PEG-A (▲) with 4arm-PEG-SH. The study was made with six samples in each group, and the error bars were too small to be seen in most of the data points (X means that the gels were too weak to be weighed.)

behavior of the gels was illustrated in Figure 4. The PEG-acrylate-based gels continued to swell until the point of ultimate gel disintegration, due to the hydrolysis of its ester bond and breakdown of the crosslinking points. In contrast, the α-PEG-MA-based gels reached the equilibrium swelling after about 3 days and no significant further swelling was observed.

To further compare the hydrolysis stability, accelerated hydrolysis experiments (with 0.2 M NaOH solution) were done with gels formed between 4arm-PEG-SH and PEG-diacrylate (PEG-A), PEG-dimethacrylate (PEG-MA), and our proposed new structure α-PEG-MA. The results (Supporting Information Fig. S3) showed that PEG-MA gel was more stable than the PEG-A gel, but our new PEG systems are much more resistant to hydrolysis than both of the other two.

CONCLUSIONS

An α-PEG-MA structure was proposed and synthesized through a one-step reaction with high yield (>90%). This new PEG derivative reacts with thiols in the Michael-type addition reaction with reactivities comparable with the conventionally-used acrylated PEG. More importantly, the linkages of the addition product are hydrolysis-stable and, hence, suitable for long-term applications such as vitreous replacement and implanted device surface coating. It should be pointed out that this newly proposed structure is not only applicable to double- and mono-functionalized PEG, but also to other polymers with hydroxyl groups such as poly(vinyl alcohols) and polysaccharides.

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