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BIODEGRADABLE ULTRA-pH SENSITIVE POLYMERS AS NANOMEDICINES FOR CANCER THERAPY

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ABSTRACT

Biodegradable ultra-pH sensitive (UPS) copolymers were prepared in the presence of a cyclic ketene acetal, 2-methylene-4-phenyl-1,3-dioxolane (MPDL), as a comonomer for the atom transfer radical polymerization (ATRP) of acrylic monomers. Due to the incorporation of ester linkages in the polymer backbone, the new nanoprobes were prone to hydrolysis and can be degraded into non-cytotoxic compounds. Meantime, with a number of ionizable tertiary amine side groups, the synthesized block copolymers displayed varied hydrophilicity at different pH. A slight pH decrease could lead to a rapid dissociation of the micelles into unimers, resulting in the release of entrapped drug or the activation of suppressed fluorophores. The detailed polymer synthesis, ultra-pH sensitivity and degradation of the new UPS nanoprobes will be discussed in this study.

Keywords: ultra-pH sensitive, micelle, tumour imaging, drug delivery, degradation, cyclic ketene acetal, ATRP.

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INTRODUCTION

Stimuli-responsive polymer-based materials have been intensively studied over the years in a wide range of applications including coatings, tissue engineering, biosensing, self-healing, and drug delivery.[1-7] pH, regarded as one of the most important stimuli in physiological systems, often plays a critical role in maintaining cellular and tissue homeostasis.[8,9] It is worth noting that the dysregulated pH has been described as a universally diagnostic hallmark of cancer.[10,11] Therefore, pH-sensitive nanoparticles that are capable of altering their chemical or physical properties have been widely developed for tumour imaging and cancer-targeted drug delivery.[12-15]

Recently, a series of ultra-pH sensitive (UPS) nanoprobes with a narrow pH responsive span were designed, which can sharply respond to and amplify *in vivo* pH signals.[16,17] The UPS nanoparticles consist of an amphiphilic block copolymer, namely PEG-*b*-PMA, where PEG is poly(ethylene glycol) and PMA is a polymethacrylate block with multiple ionizable tertiary amines. At physiological pH (7.4), the copolymers self-assemble into core-shell micelles. When pH decreases to the acidic tumour pH (6.5-6.9), tertiary amines get protonated and the micelles dissociate into "free" cationic unimers. With fluorophores conjugated on the PMA segment, the UPS copolymers can display an "all-or-nothing" fluorescence signal, providing great promise in tumour detection and image-guided surgery. The principle of this reversible micellization is described in Figure 1. Mechanistic investigation suggests that the hydrophobic micellization results in the sharp pH response that is absent in commonly used small molecular and other polymeric pH sensors.[18-20]

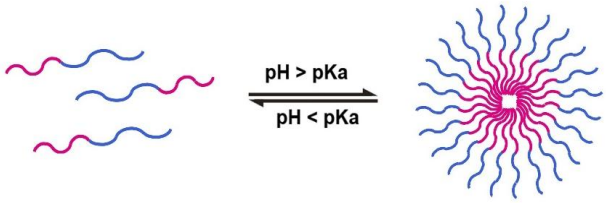


Figure 1: Reversible micellization of UPS copolymers (blue: PEG block; pink: PMA block).

Despite the high performance in terms of pH responsivity and sensitivity, the UPS nanoprobe design is severely limited in its application due to the non-biodegradable polymer backbones, where the PMA segment is synthesized by means of ATRP of acrylic monomers. It is widely accepted that a desire candidate as biomaterials, especially for drug delivery, should consist of biocompatible and biodegradable polymers[21-23] that can be degraded into non-cytotoxic compounds and eliminated from the body via metabolism. Herein, we propose the design and preparation of biodegradable UPS (designated: UPS2) copolymers, employing radical ring opening polymerization (rROP)[24,25] to incorporate ester linkages into the polymer backbones. A cyclic ketene acetal, 2-methylene-4-phenyl-1,3-dioxolane (MPDL), was synthesized and copolymerized with dialkylaminoethyl methacrylates under the ATRP conditions. The resulting copolymers with a certain amount of MPDL inserted in the backbones not only retained the ultra-pH sensitivity, but also manifested significant degradability by hydrolysis, which offered great opportunity for intracellular drug delivery and tumor-targeted imaging and therapy.

MATERIALS AND METHODS

Materials

MPDL, 2-(dibutylamino)ethyl methacrylate (DBA-MA) and PEG macroinitiator (PEG_{113}-Br) were prepared according to the procedures in the literatures.[26,27] All other chemicals and solvents were used as received from Sigma-Aldrich or Fisher Scientific Inc.

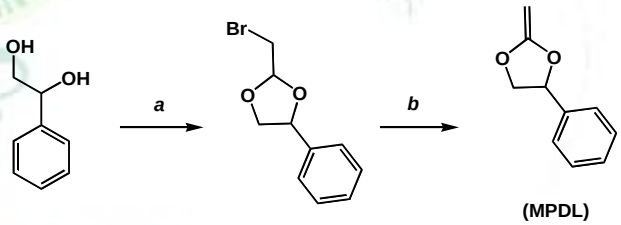
Methods

Preparation of UPS copolymers: PEG_{113}-*b*-$PDBA_{70}$ is used as an example to illustrate the procedure. DBA-MA (1.69 g, 7.0 mmol), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 21 µL, 0.1 mmol), and PEG_{113}-Br (0.5 g, 0.1 mmol) were charged into a tube, after which a mixture of isopropanol (IPA, 2 mL) and dimethylformamide (DMF, 2 mL) was injected to dissolve the monomer and initiator. Three cycles of freeze-pump-thaw were performed to remove oxygen. Then CuBr (14 mg, 0.1 mmol) was added quickly, followed by another deoxygenization cycle. The polymerization was carried out at 40 °C for 8 hours. After polymerization, the reaction mixture was diluted with 10 mL tetrahydrofuran (THF), and passed through a neutral Al_2O_3 column to remove the catalyst. THF was removed by rotary evaporator, and the residue was dialyzed in deionized water and lyophilized to yield a white powder. The final copolymer was characterized by ^{1}H 500 MHz NMR and GPC (Viscotech GPCmax, PLgel 5µm MIXED-D columns by Polymer Labs, THF as eluent at 1 mL/min).

Preparation of UPS2 copolymers: PEG_{113}-*b*-P(DBA_{50}-*r*-$MPDL_5$) is used as an example. In addition to DBA-MA (1.69 g, 7.0 mmol), PMDETA (21 µL, 0.1 mmol) and PEG_{113}-Br (0.5 g, 0.1 mmol), MPDL (0.08 g, 0.5 mmol) was also charged into the polymerization tube. The following polymerization, purification and characterization were using the same procedure as mentioned above.

RESULTS AND DISCUSSION

As a highly effective cyclic ketene acetal for radical ring-opening polymerization, MPDL was efficiently synthesized through a two-step reaction.[28] As shown in Scheme 1, styrene glycol was first reacted with bromoacetaldehyde dimethyl acetal in the presence of DOWEX H^+ resin as a catalyst for the transesterification. Since the reaction was reversible, methanol was allowed to evaporate during the reaction to achieve a higher conversion of the starting compounds. After that, the elimination reaction was carried out by treating the cyclic bromoacetal with two folds of potassium *tert*-butoxide and a catalytic amount of Aliquat® 336. The NMR spectrum in Figure 2 confirms the final product MPDL in a very high purity. Without additional purification, this cyclic ketene acetal was readily available for the following radical polymerizations.



Scheme 1: Synthetic pathway to MPDL. Reagents: (a) DOWEX H+, bromoacetaldehyde dimethyl acetal; (b) *tert*-BuOK, Aliquat® 336, dry THF.

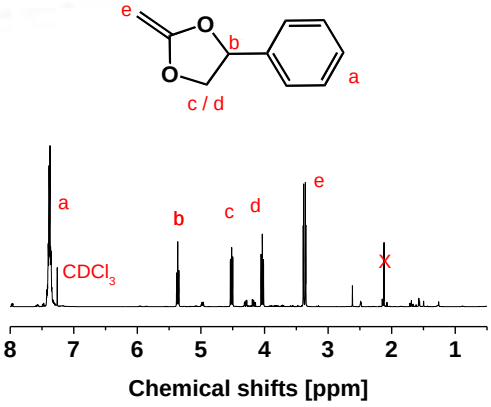
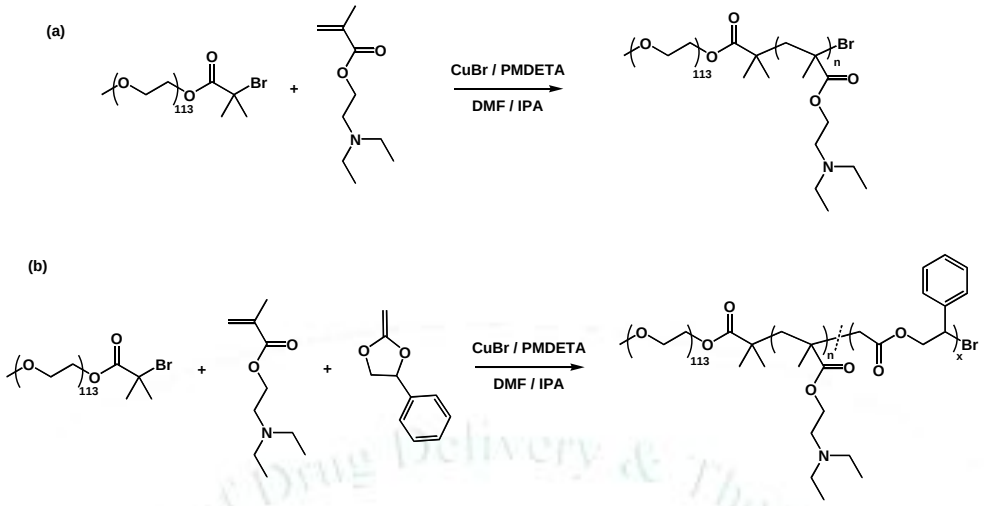


Figure 2: ^{1}H-NMR spectrum of MPDL in $CDCl_3$.

Both traditional UPS and biodegradable UPS2 copolymers were prepared by the ATRP method, as shown in Scheme 2. With α-bromoisobutyryl functionalized PEG as a macroinitiator, a series of dialkylaminoethyl methacrylates were successfully

polymerized, and the profile of these polymers was summarized in Table 1. The terms: PDEA, PDPA and PDBA were short for diethyl-, dipropyl- and dibutyl-substituted poly(aminoethyl methacrylate), respectively. Due to the incorporation of MPDL, the numbers of tertiary amine unites in UPS2 copolymers were slightly less than those in UPS copolymers, but the ultra-pH sensitivity was not affected significantly. In Figure 3, the titration curves indicated that both UPS and UPS2 copolymers had strong buffering capability in the range of 10% to 90% protonation degree, which allowed for a rapid dissociation of the polymer micelles in response to a very small pH decrease. It is also interesting to note that a longer hydrophobic chain length resulted in a lower pK_a as well as a sharper pH transition in both cases.



Scheme 2: Syntheses of PDEA (a) and PDEA* (b).

Table 1: Profile of UPS and UPS2 copolymers

Entry	Polymer name	Abbreviation	M_n (g/mol)	M_w/M_n	DP_n
1	PEG_{113}-*b*-$PDEA_{70}$	PDEA	18,800	1.26	68
2	PEG_{113}-*b*-$PDPA_{70}$	PDPA	19,500	1.19	69
3	PEG_{113}-*b*-$PDBA_{70}$	PDBA	23,700	1.25	66
4	PEG_{113}-*b*-P(DEA_{50}-*r*-$MPDL_5$)	PDEA*	15,300	1.63	49
5	PEG_{113}-*b*-P(DPA_{50}-*r*-$MPDL_5$)	PDPA*	16,500	1.59	48
6	PEG_{113}-*b*-P(DBA_{50}-*r*-$MPDL_5$)	PDBA*	20,700	1.44	46

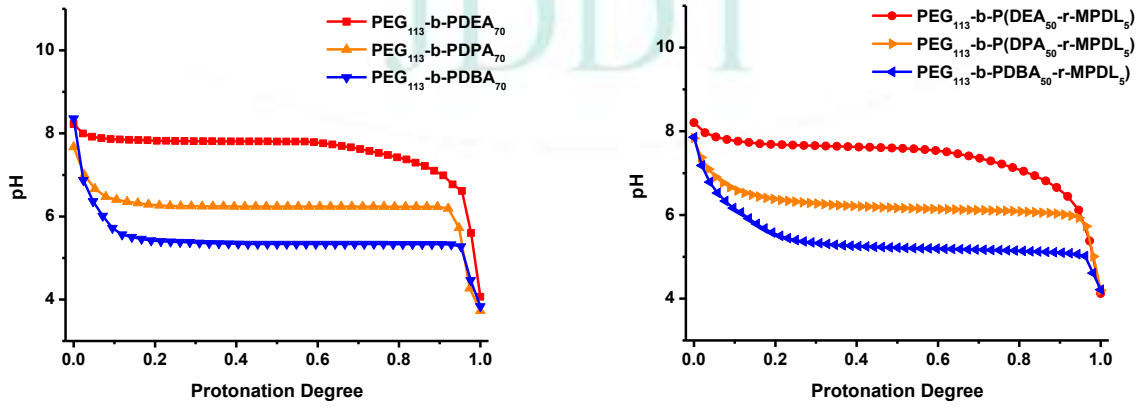


Figure 3: Titration curves of UPS and UPS2 copolymers.

To further demonstrate the ultra-pH sensitivity of biodegradable UPS2 copolymers, hydrodynamic polymer size was determined at different pHs by dynamic light scattering (DLS). Figure 4 describes the results of three UPS2 copolymers in PBS buffers. Based on the above-mentioned titration, the pK_a of PEG_{113}-*b*-P(DEA_{50}-*r*-$MPDL_5$) (PDEA*), PEG_{113}-*b*-P(DPA_{50}-*r*-$MPDL_5$) (PDPA*) and PEG_{113}-*b*-P(DBA_{50}-*r*-$MPDL_5$) (PDBA*) were 7.6, 6.2 and 5.1, respectively. As for PDEA*, micelle formation was observed only in the buffer with pH at 8.07. Interestingly, a relatively larger size than expected was found in buffer 6.74 (pH 6.74), which was possibly due to the uncomplete dissociation of the "not ultra-enough" PDEA* micelles. No surprisingly, this phenomenon did not apply to PDPA* and PDBA*, which contained longer alkyl substitutions.

For the former, the polymer size decreased sharply from buffer 6.74 to 5.86, indicating the rapid dissociation of the micelles into unimers. The latter showed a more dramatic breakdown when pH was changed from 5.86 to 4.85. The DLS results illustrated that all these three UPS2 copolymers, especially PDPA* and PDBA*, possessed of significant ultra-pH sensitivity. However, considering the little difference between physiological pH (7.4) and slightly acidic tumour pH (6.5-6.9), a new type of UPS2 copolymer should be designed with a pK_a at 7.1-7.2, in which the polymer preserved the micellar conformation in normal cells but spontaneously turned into unimers in tumour cells. A simple and practical strategy can be used by copolymerizing DEA and DPA monomers, or directly polymerizing another methacrylate with a hybrid (ethylpropyl) substitution.

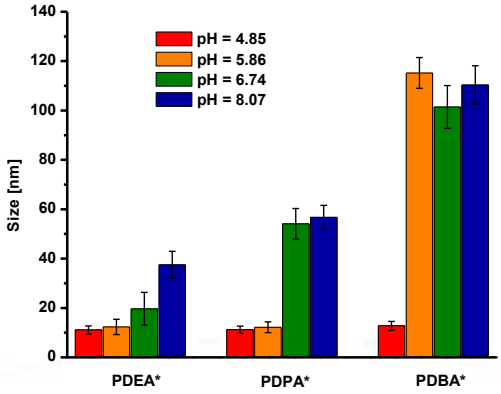


Figure 4: Particle sizes of UPS2 copolymers at difference pHs.

In order to examine the biodegradability of the UPS2 copolymers, a hydrolytic study was carried out with PDBA*. An accelerated hydrolytic condition was used for the degradation; that was in THF with 5% potassium hydroxide (KOH) in methanol. The molecular weight of PDBA* was monitored by gel permeation chromatography (GPC) during the hydrolysis. As shown in Figure 5(PDBA*), the molecular weight decreased from 20,700 g/mol to 8,800 g/mol after 1 d, along with a narrowed molecular weight distribution. It is known that esters are prone to hydrolysis in a basic or acidic environment. With the copolymerization of MPDL, a certain number of esters were incorporated into the polymer backbone randomly. During the hydrolysis, the ester linkages broke and the PMA block was cleaved into several oligomer chains, causing an apparent decrease in the molecular weight. In comparison, a traditional UPS copolymer with a similar chain length, PDBA, was hydrolyzed in the same condition as a control. It can be seen in Figure 5(PDBA), the molecular weight decreased by *ca.* 6,000 g/mol after 1 d hydrolysis, which was possibly due to the detachment of PEG from the PMA block. The alike molecular weight distributions also indicated that the PMA segment was able to preserve even in such a harsh condition.

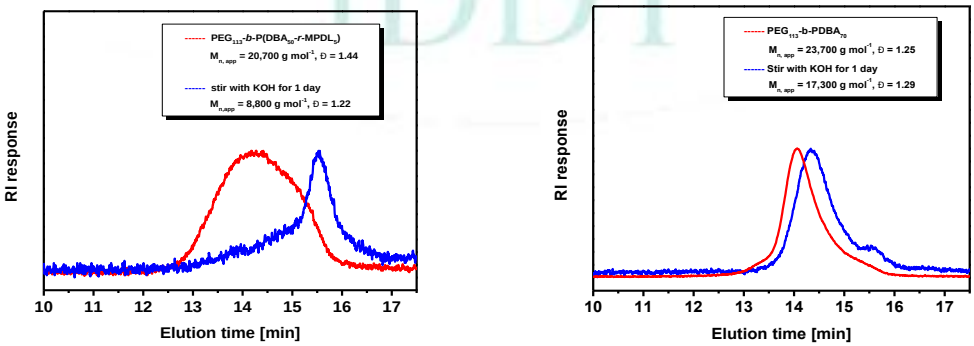


Figure 5: Hydrolysis study using GPC (left: PDBA*; right: PDBA).

CONCLUSION

In this study, we have efficiently synthesized a cyclic ketene acetal, MPDL, which is able to confer biodegradability to vinyl polymers by a radical ring-opening mechanism. Under the ATRP conditions, MPDL was copolymerized with a series of dialkylaminoethyl methacrylates in the presence of PEG as a macroinitiator, affording biodegradable block copolymers with a number of tertiary amine side groups. Based on the combination of ATRP chemistry and rROP of MPDL, biodegradable UPS polymers were successfully synthesized, and their ultra-pH sensitivity and degradability were systematically studied. This work opens up new pathways towards the design of bio-related stimuli-responsive polymers, and the development of biodegradable UPS copolymer nanoprobes are undoubtedly of great potential in both current and future cancer therapy.

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