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Facile Synthesis of Well-defined Hydrophilic Polyesters as degradable Poly(ethylene glycol)-like Biomaterials†

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Highly stable and polymerizable *δ*-valerolactones bearing oligo(ethylene glycol) methyl ether functionalities are facilely prepared by alkylphosphine catalyzed thiol-ene addition with an exocyclic *α, β*-unsaturated *δ*-valerolactone. The functionalized lactones undergo efficient ring-opening polymerization (ROP) to afford well defined PEG-like polyesters. Kinetic studies revealed that the ROP, catalyzed by diphenyl phosphate at ambient temperature, bears living characters. The results of cell viability essays indicate that the resultant polyesters are fully biocompatible. *In vitro* tests on protein adsorption and cell adhesion demonstrate that the antifouling capability of these polyesters is comparable to that of the PEG. The strategy of facile preparation of stable and polymerizable lactones bearing functional substituents reported here provides a versatile platform for the development of polyester-based new biocompatible and biodegradable polymeric materials for biomedical applications.

Poly(ethylene glycol) (PEG) is a neutral, hydrophilic, and biocompatible polyether that is widely adopted in biomedical applications. PEG is well known as one of the most effective synthetic polymers in reducing non-specific protein adsorption.[1,2] PEGylzation has also been extensively exploited on a wide variety of chemical and biomedical entities, including small drugs and pharmaceutical carriers, in order to enhance their biomedical efficacy and physicochemical properties.[3] In fact, PEG is the only synthetic polymer approved by Food and Drug Administration (FDA) for preparing polymer-protein conjugates.[4,5] However, with rapidly growing interest in protein-based therapeutics,[6] the inherent nonbiodegradability of PEGs, as one of the major drawbacks, has raised more and more concerns.[7,8] High-molecular-weight (high-M_w) PEGs (over 40 kDa) are metabolically inert, with their excretion rates being significantly reduced.[9] Recently, an increasing number of reports show that high-M_w PEGs can accumulate and cause vacuolation in the liver, kidney, spleen and tissues after administration.[7b-7e,10] Growing concerns of bioaccumulation and cytoplasmic vacuolization issues of PEG prompt research for solutions to overcome its limitation of the non-biodegradability. One solution is to incorporate cleavable moieties into the backbone of PEG based on the step-growth polymerization.[11,12] However, linear PEG-analogues offered by these approaches are usually not well defined. Many biomedical applications, especially *in vivo* ones, would benefit from synthetic polymeric materials with well defined structures (e.g. narrow polydispersity, PDI).[13]

Well defined polymethacrylates with cleavable pendent oligomeric poly(ethylene oxide) (PEO) side chains as degradable PEG analogues have been synthesized via controlled radical polymerization,[14-17] but their non-biodegradable carbon-carbon backbones could might limits their *in vivo* applications as biomedical materials.[18] Aliphatic poly(ester)s, on the other hand, offer backbones that are biocompatible and biodegradable via either hydrolytic or enzymatic pathways,[19] making them one of promising candidates for the development of backbone degradable PEG analogues. One efficient strategy of preparing well defined functional polyester is by controlled ring-opening polymerization (ROP) of functionalized lactones. Though several strategies have been reported to obtain polyester with hydrophilic ether side chains by ROP of functionalized lactones,[20,21] facile access to functionalized lactones that undergo controlled ROP remains quite challenging, considering the particularly labile nature of the lactone structures.

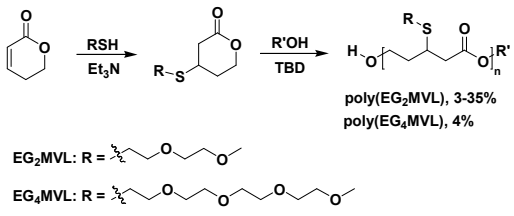

Figure 1. Previously reported synthesis of PEO functionalized valerolactones and their subsequent organocatalytic ROP.[22]

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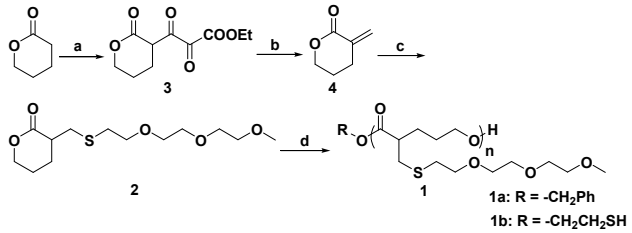
Recently, Waymouth and co-workers reported an elegant strategy for installing PEO pendent groups in the lactone ring via thiol-ene Micahel addition with an endocyclic *α, β*-unsaturated *δ*-valerolactones and their subsequent ROP to afford PEG-like hydrophilic polyesters (Figure 1)[22] though the biocompatibility and antifouling ("stealth") capability of these PEG-like polyester were not evaluated in the paper. This strategy with advantageous features such as very short synthetic route for lactone functionalization and absence of metal catalysts in the whole process made it very attractive as a platform for preparation of polyester-based PEG-like biomedical materials. Unfortunately, as noticed by the authors, the yields of the functionalized monomers were moderate (44-54%) and, more importantly, the ROP processes were not efficient at all as evidenced by low yields (3-35%, homopolymer), which made this approach almost unpractical. The authors suggested that the observed low efficiency of the ROP could be due to the unfavorable thermodynamics of the ring-opening of the substituted *δ*-valerolactones. Meanwhile, the authors reported that the thiol adducts of the endocyclic *α, β*-unsaturated *δ*-valerolactones were unstable and tended to lose thiol groups by elimination through the so called retro-Michael mechanism.[22,23] Therefore, it is reasonable to hypothesize that the instability of the products of thiol-ene Micahel addition could account for the moderate yield of the thiol-ene addition and might complicate the subsequent ROP processes, resulting in the observed low efficiency of the ROP. To test our hypothesis, we need to find a way to greatly enhance the stability of the thiol adducts of *α, β*-unsaturated *δ*-valerolactones. It is noticed that Kupchan and co-workers reported that thiol adducts of exocyclic unsaturated *γ*-butyrolactones were considerably more stable than those of their endocyclic counterparts due to steric and inductive effects.[24] Despite the different ring size of *δ*-valerolactone and *γ*-butyrolactone, it is highly likely that the same principle of thiol-ene addition of unsaturated *γ*-butyrolactone may also be applicable to unsaturated *δ*-valerolactones. Put together, we envision that the thiol-ene adducts of exocyclic *α, β*-unsaturated *δ*-valerolactones should be more stable than their exocyclic counterparts, which will lead to more efficient thiol-ene addition and subsequent ROP of the adducts.

Herein, we report the preparation of highly stable and polymerizable *δ*-valerolactones bearing PEO functionalities. The resultant lactones efficiently undergo controlled ROP to afford well defined PEG-like polyesters. The results of cell viability essays indicate that the resultant polyesters are fully biocompatible. *In vitro* tests on protein adsorption and cell adhesion demonstrate that the antifouling capability of these polyesters is comparable to that of the PEG.

To test our hypothesis, exo-methylene cyclic lactone **4** was designed (Scheme 1). Exocyclic lactone **4** was prepared from *δ*-valerolactone in two steps with good yield (68%) based on similar procedures reported.[25] The PEO pendent groups derived from triethylene glycol dimethyl ether (TGDME) are introduced to lactone **4** through thiol-ene addition catalyzed by tributylphosphine,[26] to give PEO-functionalized lactone monomer **2** in excellent yield (85-95%) and the monomer **2** is found to be stable on bench top for months. These results clearly confirm one of our hypothesis, i.e., the thiol-ene adducts of exocyclic unsaturated *δ*-valerolactones are more stable than their endocyclic counterparts,[22] which indeed leads to more efficient formation of the thiol-ene adducts.

Scheme 1. Synthesis of PEO functionalized lactone monomer **2** and ring-opening polymerization[a]



[a] Reagent and conditions: (a) Ethanol, diethyl oxalate, sodium metal, 0℃; (b) THF, NaH, CH_2O, rt, 30 minutes; (c) CH_2Cl_2, RSH, rt, 30 minutes; (d) ROH, diphenyl phosphate, rt, 24 hours.

ROP of monomer **2** was then investigated. Due to intended biomedical applications, organocatalysts for ROP are preferred over metal-based catalysts which often leave trace amounts of metal residue in the polymer products. After screening a number of organocatalysts (data not shown), diphenyl phosphate (DPP)[27] was found to be the most effective for the ROP of functional lactone monomer **2**. It was found that the cationic ROP of **2**, with benzyl alcohol as the initiator, lead to high monomer conversion with different monomer to initiator feed ratio at ambient temperature (Table 1). Conversion was estimated by integration of the ^{1}H NMR spectral signals at *δ* 4.27 (-CH_2O- in lactone ring of the monomer) and *δ* 4.11 (-CH_2O- in the polymer backbone) (Figure 2 and Figure S1).

Table 1. DPP Catalyzed Ring-Opening Polymerization of monomer **2**

run	$[M]_0/[I]_0$[a]	Conv[b] (%)	Yield[c] (%)	$M_{n,NMR}$[d]	M_n[e]	M_w/M_n[e]
1	20	89	73	5364	5214	1.01
2	50	82	78	12080	10144	1.16
3	80	79	76	16755	14191	1.12
4	100	83	75	23760	21027	1.12

[a] Room temperature; benzyl alcohol as initiator; [I]/[DPP] = 1:1; 24 hours. [b] Measured by ^{1}H NMR spectroscopy. [c] Isolated yield after purification by dialysis. [d] Determined based on ^{1}H NMR end-group analysis. [e] Determined by GPC with polystyrene as standards.

Notably, according to ^{1}H NMR spectra of the polymer of monomer **2**, the methylene protons of initiator (*BnOH*) appeared in the range of *δ* 5.12 (c) and methylene protons of polymer chain end adjacent to OH group (-CH_2-*OH*) are observed at *δ* 3.46 (b). The ratio of integrals of signals (c) and (b) is found to be close to 1/1, which suggests that all polymer chains might have been initiated from benzyl alcohol. GPC analysis shows that the obtained polymers

have relatively narrow polydispersity (1.01-1.16), which suggests the living nature of the polymerization processes. To provide

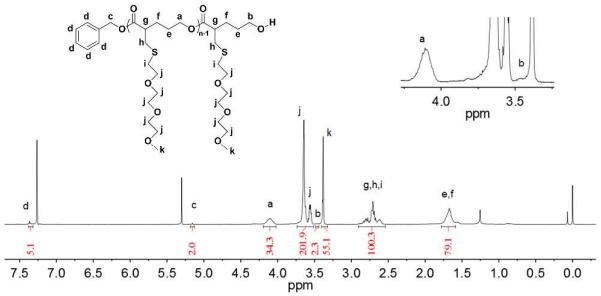


Figure 2. ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of a polymer obtained by polymerization of monomer **2** with BnOH as initiator (CH_2Cl_2, 25^{o}C, $[M]_0/[BnOH]_0/[DPP]_0$ = 20/1/1, [M] = 10 M).

additional insights, the kinetics of the ROP of monomer **2** is investigated. Aliquots taken from the reaction mixture at different time intervals during the polymerization reaction (run 3, Table 1) were immediately treated with basic alumina to remove the catalyst and thus to quench the reaction. The samples are then concentrated and analyzed by ^{1}H NMR and GPC (Table S1). It was observed that the molecular weight (M_n) of the polymer products increased linearly with monomer conversion, in good agreement with the theoretical values (especially at medium molecular weights), while the polydispersity indices remained narrow (M_w/M_n < 1.16) up to high monomer conversion (Figure 3a).

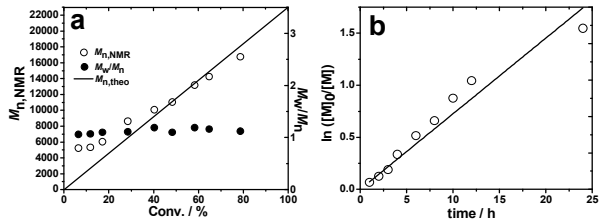


Figure 3. (a) Dependence of $M_{n,NMR}$, polydispersity (M_w/M_n) on conversion (conv.). The line shows the theoretical $M_{n,theo}$ calculated from the equation ($[M]_0/[BnOH]_0$) × conv. × M_w of **2** + (M_w of BnOH). Polydispersity was determined by GPC in THF with polystyrene as standards. (b) Kinetic plots of polymerization of **2**.

The linear dependence of ln($[M]_0/[M]$) on polymerization time shown in Figure 3b demonstrates a clear first-order kinetics on monomer concentration, indicating that the rate of monomer consumption remains constant during the polymerization. These observed linear relationships have established the controlled nature of the cationic ROP of monomer **2**. Taken together, the observed efficient thiol-ene adducts formation and subsequent ROP confirms the other hypothesis that stability of thiol-adducts of unsaturated lactones plays very important role in determining the efficiencies of ROP of the resultant PEO substituted δ-valerolactones.

With well defined PEG-like polyesters in hands, we set out to assess the antifouling capabilities of these materials. To test its resistance to protein adsorption and cell adhesion, polymer **1b** (M_n = 5,364 Da, PDI = 1.01) with a terminal thiol group is conveniently prepared using 3-mercaptopropan-1-ol as the initiator (Scheme 1 and S1). Polymer **1b** was then immobilized on gold surface, forming a self-assembled monolayer (SAM) that is subjected to analysis with quartz crystal microbalance (QCM) to estimate the extent of nonspecific adsorption of protein molecules to its surface. SAMs of *n*-hexadecanethiol and mPEG-SH (M_w = 5,000 Da) on gold are used as positive and negative controls in the QCM studies. Prior to the QCM studies, simple water contact angle tests were carried out to assess surface wettability, which gave water contact angles of 48.7° for polymer **1b**, 31.8° for *m*PEG-SH and 103.5° for *n*-hexadecanethiol SAMs (Figure S3). These results indicate that, similar to PEG, the surface modified with polymer **1b** was highly hydrophilic. For QCM studies, albumin, the most abundant protein in the circulatory system, was used as the model protein. Figure 4 shows the QCM adsorption profiles of bovine serum albumin (BSA) onto the surfaces modified by SAMs of mPEG-SH (5,000 Da), *n*-hexadecanethiol, and polymer **1b** (5,364 Da, PDI = 1.01). A dramatic frequency shift (114 Hz) accompanied the adsorption of BSA onto the surface modified with n-hexadecanethiol. In contrast, very small frequency shifts were observed with surfaces modified with mPEG-SH (14.3 Hz) and polymer **1b** (15.4 Hz). According to the Sauerbrey equation, the measured frequency shift (Δf) is related to the adsorbed mass per unit area (Δm) though their relationship is not directly proportional in this case.[28] Nevertheless, it is obvious that QCM adsorption profiles of BSA onto surfaces modified by polymer **1b** is very similar to that of mPEG-SH, suggesting that polymer **1b** exhibits the similar resistance to protein adsorption as PEGs.

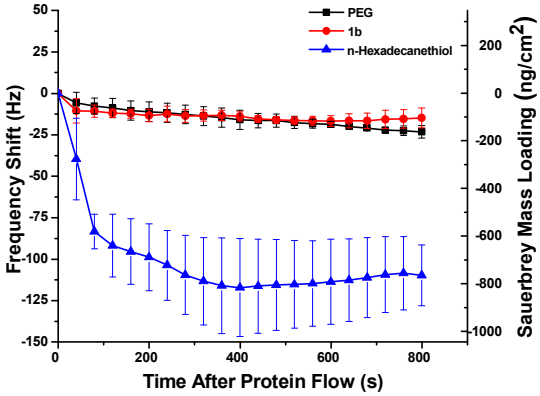


Figure 4. QCM adsorption profiles of BSA onto sensor surfaces modified by SAMs of mPEG-SH (5,000 Da), n-hexadecanethiol and **1b** (5,364 Da, PDI = 1.01). Initial protein concentration, 20 mg mL^{-1}. Data are collected in triplicate.

To further evaluate the antifouling properties of surfaces modified with polymer **1b**, cell adhesion studies are carried out with RAW 264.7 macrophage cells. Gold substrates modified with the three different SAMs are seeded with RAW 264.7cells and incubated for 24 hours in culture media. Cell attachment is assessed by live cell staining and fluorescent image analysis (Figure 5). It is found that the substrates modified by the SAM of polymer **1b** and *m*PEG-SH shows high resistance to cell adhesion. As demonstrated by the plots of adhered cell density (Figure 5d), the abilities of PEG and **1b** to resist cell adhesion are fully comparable. In contrast, the

surface modified with *n*-hexadecanethiol shows considerably high cell adhesion, presumably due to the highly hydrophobic nature of this surface. In addition, the cytotoxicity of polymer **1b** is evaluated with cell viability assay based on 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT).[29] The obtained results clearly show that, similar to *m*PEG-SH, polymer **1b** is not toxic to human foreskin fibroblast (HFF) cells in the concentration range (0.2-1 mg/mL) examined (Figure S6).

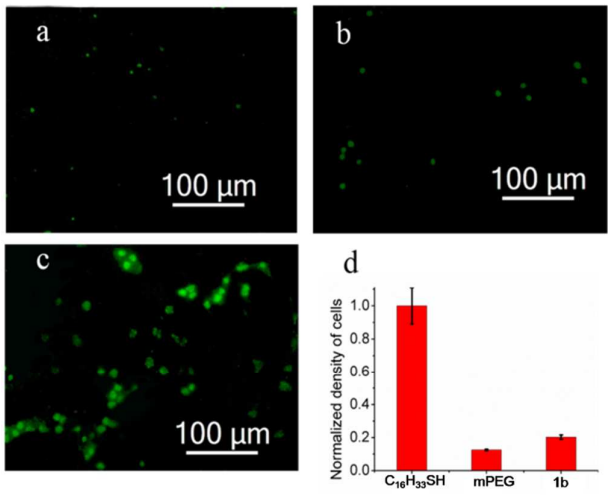


Figure 5. Microscopic images of attached RAW 264.7 cells stained with Calcein on gold surfaces modified by SAM of mPEG (5,000 Da, a); **1b** (5,364 Da, PDI = 1.01, b) and hexadecanethiol (c). (d) The density of the adhered cells. Data are collected in triplicate.

In summary, our results have demonstrated that the thiol-ene addition is a powerful tool in introducing functionalities to labile lactone structures and also revealed that the stability of thiol-adducts of unsaturated lactones plays predominant roles in the formation of the thiol adducts and in the subsequent ROP processes. In contrast to that of endocyclic unsaturated *δ*-valerolactones, the PEO substituted *δ*-valerolactones from exocyclic ones are more stable and undergo efficient ROP in a well controlled manner. QCM and cell adhesion studies have revealed that the resultant well defined PEG-like polyester has excellent protein-resistant and anti-cell adhesion capabilities. In fact, the observed antifouling properties of surfaces modified by this PEG-like polyester are comparable to those of PEG with similar molecular weight. Further studies are needed to reveal the exact mechanism of the observed anti-fouling properties of these hydrophilic polyesters. Nevertheless, the strategy of facile preparation of stable and polymerizable lactones bearing functional substituents reported here provides a versatile platform for the development of polyester-based new biocompatible and biodegradable polymeric materials for biomedical applications.

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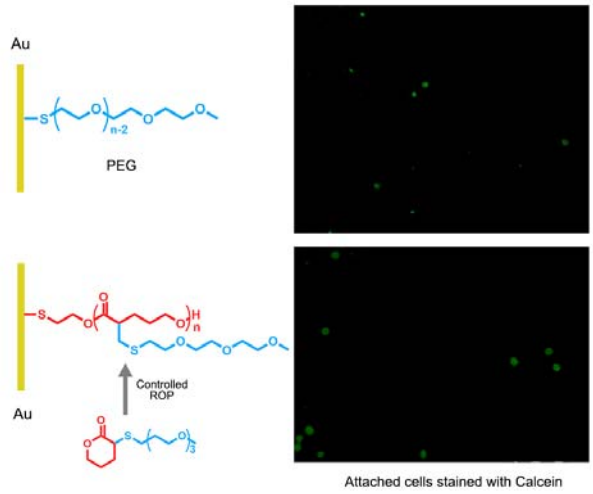

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