Supporting Information

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

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1. Materials and Methods

Chemicals were purchased from commercial sources and used as received. Silica gel for analytical thin layer chromatography (TLC) and column chromatography (200–300 mesh) were purchased from Qingdao Haiyang Chemical Co., Ltd & Special Silica Gel Factory (Qingdao, China). The $^1$H NMR spectra were recorded at 400 MHz and $^{13}$C NMR spectra were measured at 100 MHz on a Bruker AV400 spectrometer at ambient temperature. Chemical shifts were reported in parts per million (ppm) downfield from TMS (tetramethylsilane). Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) was performed on a Bruker Reflex III with a UV laser operating at 337 nm and an accelerating voltage of 20 kV. 1, 8, 9-trihydroxyanthracene (dithranol) was used as matrix. Samples were dissolved in THF (10 mg/mL) and mixed with matrix (20 mg/mL in THF) at a mixing ratio of 20 : 5 (v/v, matrix : analyte). ESI mass spectra were performed on a Bruker microTOF-Q II instrument. Gel Permeation Chromatography (GPC) was carried out using a PL-GPC 50 Integrated GPC/SEC System with a RI detector (Agilent Technologies, Inc. USA) with polystyrene as standards (American Polymer Standards Corp, USA). The eluant was tetrahydrofuran (THF) and flow rate was set to 1 mL/minute at 35°C. Dialysis was carried out with tubing cellulose membrane (MWCO 1KD, Spectrum Laboratories Inc. USA). Preparation of Mixed Self-Assembled Monolayers (SAMs): The Au substrates and QCM Crystals were cleaned by immersion in piranha solution (3:1, H$_2$SO$_4$ : 30% H$_2$O$_2$) at room temperature for 10 min, rinsing with Ultra Pure water and then HPLC grade EtOH thoroughly for 1 min, and then dried under N$_2$ atmosphere. The treated Au substrates and QCM Crystals were immersed in EtOH solution of the polymer 1b (5364 Da, PDI = 1.01), mPEG-SH and n-Hexadecanethiol for 24 h, and then rinsing with EtOH, followed by drying under N$_2$ atmosphere. Water contact angle measurements were performed with a video-based optical contact angle measuring instrument (Dataphysics OCA 15EC, DataPhysics Instruments GmbH, Germany). Quartz crystal
microbalance (QCM) assays were carried out using a CHI 420C electrochemical analyzer (Chenhua Instruments, Shanghai, China). The phosphate buffered saline (phosphate 200 mM, pH 7.4, NaCl, 100 mM) used in QCM measurements was prepared at the time of use. Thermal transition data were collected with a Mettler differential scanning calorimeter (DSC) (STAR® System, Mettler Toledo, Switzerland) equipped with a liquid nitrogen cooling system, calibrated with indium standards. A dry and constant flow of nitrogen (40 mL min⁻¹) was maintained in order to eliminate thermal gradients and ensure the validity of the calibration standard from sample to sample. The sample size ranged from 5 to 8 mg, and each sample was subjected to two cool-heat-cool cycles from -150 °C to 0 °C with a heating and cooling rate 10 °C/min.

HFF cells and Raw 264.7 cells were obtained from Cell Resource Center (IBMS, CAMS/PUMC) and cultured in Dulbecco’s Modified Eagle Medium (DMEM; Thermo Scientific) supplemented with 10% fetal bovine serum (FBS; GIBCO; Invitrogen), and 1% penicillin/streptomycin (Beijing Solarbio Scientific & Technology Co, Ltd). For imaging studies, cells were plated in Class Bottom Cell Culture Dish (Nest) containing 1 mL of complete DMEM and incubated at 37°C under 5% CO₂ for one day. Bright filed and fluorescence images were taken with a Zeiss Abserver A1 inverted fluorescence microscope equipped with an EM-CCD camera (Hamamatsu) and an X-Cite 120 metal halide lamp (EXFP). Bright field image and fluorescence images were obtained using a 40×objective lens.
2. Synthesis and Characterizations

Scheme S1. (a) Ethanol, diethyl oxalate, sodium metal, ice/salt bath 2 h, rt, overnight; (b) THF, NaH, CH₂O, rt, 30 minutes; (c) CH₂Cl₂, 7, rt, 30 minutes; (d) ROH, diphenyl hydrogen phosphate, rt, 24 hours.

Synthesis of Monomer

Ethyl 2-oxo-2-(2-oxo-tetrahydro-2H-pyran-3-yl)acetate (3)¹. Sodium metal (3.45 g, 0.15 mol) was cautiously added to 100 mL absolute ethanol. When the solid disappeared, the solution was cooled by an ice/salt bath, and diethyl oxalate (16.06 g, 0.11 mol) was added to the solution. δ-valerolactone (10.00 g, 0.1 mol) dissolved in 20 mL ethanol was added dropwise about 20 minutes. The reaction mixture was stirred at -15°C for 1 hour. Upon removal of the cooling bath, the solution was allowed to warm to room temperature and stirred for 10 hours. The solvent was then removed under reduced pressure. The pasty residue was diluted with 100 mL H₂O, and the aqueous phase was washed with diethyl ether (50 mL) and the organic phase was washed by H₂O (50 mL). The combined aqueous phase was acidified with dilute HCl (2 M), and extracted with DCM (50 mL × 3). The combined organic phases were dried with anhydrous Na₂SO₄, and the solvent was removed under reduced pressure to
give 3 as yellow oil which was used in the next step without any further purification.

![Image](image_url)

**3-Methylene-tetrahydropyran-2-one (4)**. Ethyl 2-oxo-2-(2-oxo-tetrahydro-2H-pyran-3-yl)acetate (3, 1.00 g, 0.05 mol) dissolved in 10 mL dry THF was added dropwise to a suspension of degreased NaH (1.20 g, 0.05 mol) in 10 mL dry THF. The reaction mixture was stirred at ambient temperature till H₂ evolution ceased. Anhydrous gaseous formaldehyde (generated by thermal cracking of dry paraformaldehyde) was bubbled through the solution by a steam of N₂ carrier gas. After 1 hour at ambient temperature, the reaction mixture was filtered through Celite to remove sodium oxalate and formaldehyde polymer, and the solvent was removed from the filtrate under reduced pressure. The residue was dissolved in 30 mL DCM, and washed with saturated KHCO₃, and the organic phase was dried with anhydrous Na₂SO₄, and the solvent was removed under reduced pressure. The isolated residue was purified by column chromatography (SiO₂, eluent: EtOAc/n-hexane = 1:3) to give 3-methylene-tetrahydropyran-2-one as a colorless oil (0.38 g, 68%); ¹H NMR (CDCl₃, 400 MHz) δ 6.43(d, 1H, J = 1.2 Hz), 5.56(d, 1H, J = 1.4 Hz), 4.38(t, 2H, J = 1.4 Hz), 2.67(t, 2H, J = 6.5 Hz), 1.96(m, 2H). ¹³C NMR(100 MHz, CDCl₃) δ 165.48, 134.14, 128.25, 69.67, 28.14, 23.25. ESI MS calculated 112.1, found 135.1 (M+Na⁺).

![Image](image_url)

**2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5)**. Triethylene glycol monomethyl ether (16.40 g, 0.1 mol) and 4-toluene sulfonyl chloride (19.06 g, 0.102 mol) were dissolved in 70 mL DCM. The solution was cooled to 0°C and KOH (22.40 g, 0.4 mol) was added slowly. The reaction mixture was left to stir at ambient temperature for 16 hour, and then, poured it into ice/water and extracted with DCM.
(150 mL × 3). The organic phase was washed with H₂O and the aqueous phase was back extracted with DCM (150 mL × 3). The combined organic phases were dried with anhydrous Na₂SO₄, and the solvent was removed under reduced pressure to give 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate as a colorless oil (30.21 g, 95%); ¹H NMR (CDCl₃, 400 MHz) δ 7.80(d, 2H, J = 8.2 Hz), 7.34(d, 2H, J = 8.0 Hz), 4.16(t, 3H, J = 4.8 Hz), 3.71-3.50(m, 10H), 3.38(s, 3H), 2.45(s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 144.73, 133.10, 129.78, 127.95, 71.90, 70.73, 70.53, 69.19, 68.67, 58.98, 21.58; ESI MS calculated 318.4, found 341.1 (M+Na⁺).

S-2-(2-(2-methoxyethoxy)ethoxy)ethyl ethanethioate (6)². 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate(5, 31.83g, 0.1mol) and potassium thioacetate were dissolved in 250 mL CH₃CN and heated to reflux temperature under N₂ for 16 hours. The solvent was then removed under reduced pressure, the atropurpureus residue was diluted with 200 mL DCM, and the organic phase was washed with H₂O(100 mL), dried with anhydrous Na₂SO₄, and the solvent removed under reduced pressure. The isolated residue was purified by column chromatography (SiO₂, eluent: EtOAc/n-hexane = 1:4) to give S-2-(2-(2-methoxyethoxy)ethoxy)ethyl ethanethioate as a red oil(13.54g, 61%); ¹H NMR (CDCl₃, 400 MHz) δ 3.67-3.54(m, 10H), 3.38(s, 3H), 3.10(t, 2H, J = 6.5 Hz), 2.34(s, 3H). ¹³C NMR (100MHz, CDCl₃) δ 195.46, 71.94, 70.57, 70.54, 70.31, 69.75, 59.02, 30.52, 28.84; ESI MS calculated 318.4, found 341.1 (M+Na⁺).

2-(2-(2-Methoxyethoxy)ethoxy)ethanethiol (7)². S-2-(2-(2-methoxyethoxy)ethoxy)ethyl ethanethioate(6, 11.15 g, 0.05 mol) was dissolved in MeOH (100 mL)/aq. HCl (10%, 100 mL). The reaction mixture was heated at 100°C for 2.5 h, and then, cooled to ambient temperature, and extracted with DCM (75 mL × 3). The organic phase was
washed with saturated NaHCO₃ (100 mL), dried with anhydrous Na₂SO₄, and the solvent removed under reduced pressure. The product was further purified by vacuum distillation to give 2-(2-(2-methoxyethoxy)ethoxy)ethanethiol as a pale yellow liquid (6.84 g, 76%); ¹H NMR (CDCl₃, 400 MHz) δ 3.67-3.53 (m, 10H), 3.38 (s, 3H), 2.69 (dt, 2H, J₁ = 8.1 Hz, J₂ = 6.5 Hz), 1.58 (t, 1H, J = 8.2 Hz). ¹³C NMR (100 MHz, CDCl₃) δ 72.93, 72.00, 70.61, 70.28, 59.04, 24.29; ESI MS calculated 180.1, found 203.1 (M+Na⁺).

3-((2-(2-Methoxyethoxy)ethoxy)ethylthio)methyl)-tetrahydropyran-2-one (2). 3-methylene-tetrahydropyran-2-one (4, 1.12 g, 0.01 mol) and tri-n-butylphosphine were dissolved in 50 mL DCM. 2-(2-(2-methoxyethoxy)ethoxy)ethanethiol (7, 1.80 g 0.01 mol) dissolved in 20 mL DCM was added dropwise about 10 min. the reaction mixture was stirred at ambient temperature for 30 minutes. The solvent was then removed under reduced pressure. The isolated residue was purified by column chromatography (SiO₂, eluent: EtOAc/n-hexane = 1:1) to give 3-((2-(2-methoxyethoxy)ethoxy)ethylthio)methyl)-tetrahydropyran-2-one as a pale yellow oil (2.39 g, 82%); ¹H NMR (CDCl₃, 400 MHz) δ 4.34 (m, 2H), 3.68-3.53 (m, 10H), 3.38 (s, 3H), 3.16-3.10 (m, 1H), 2.78-2.70 (m, 4H), 2.27 (m, 1H), 1.94 (m, 2H), 1.67 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 71.93, 70.91, 70.61, 70.57, 70.35, 68.75, 59.05, 40.58, 34.12, 32.47, 24.17, 22.06; ESI MS calculated 292.1, found 315.1 (M+Na⁺)

**General Procedure of Polymerization.**

A typical procedure for the polymerization is as follows: In a glove box, 3-((2-(2-Methoxyethoxy)ethoxy)ethylthio)methyl)-tetrahydropyran-2-one (2, 0.162 g, 0.55 mmol) was added to a stock solution of ROH (11.2 μL, 11.2 μmol) in DCM at 25°C. A CH₂Cl₂ stock solution of diphenyl hydrogen phosphate (DPP) (11.2 μL, 11.2 μmol) were added to the solution to initiate the polymerization under an argon
atmosphere. The reaction mixture was stirred at 25°C for 24 h (until the conversion of monomer 2 monitored by $^1$H NMR spectroscopy was higher than 80%). The mixture was then treated with basic alumina in order to eliminate the catalyst and concentrated under vacuum. The mixture was dissolved in methanol (5 mL), then put in a dialysis tube (MWCO = 1000) and dialyzed against methanol (100 mL). The solvent was replaced every 12 hours. After 2 days, the polymer was then concentrated and residue solvent was removed via vacuum pump overnight.
### 3. Kinetic Study of the Polymerization

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<th>(M_{n,\text{NMR}})^(^a)</th>
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**Table S1.** Data of Figure 3. \(^a\) Determined by \(^1\)H NMR. \(^b\) \(M_{n,\text{theo}} = [M]_0/[BnOH]_0 \times \text{conv.} \times (M_w \text{ of } 2) + (M_w \text{ of } \text{BnOH})\). \(^c\) Determined by GPC in THF with polystyrene as standards (1 mL/min, 35°C). The data was collected with the reactant ratio of \([2]_0/[\text{BnOH}]_0/[\text{DPP}] = 80/1/1.\)
4. Examples of $^1$H NMR Analysis

**Figure S1.** Representative $^1$H NMR (CDCl$_3$, 400MHz) spectra of the monomer and ROP reaction mixture, entry 5 in Table S1. $M_n$-NMR = (H$_a$polymer/H$_a$monomer + 1) × ($M_w$ of 2) + ($M_w$ of BnOH); Conv. = H$_a$polymer/(H$_a$polymer + H$_a$monomer) × 100%.
5. Stability evaluation of the monomer by $^1$H NMR

Figure S2. Monomer 2 was isolated and stored on bench top in ambient environment. Samples were taken and checked with $^1$H NMR in 1(a), 8 (b) and 20 days (c).
6. The $^1$H-$^1$H COSY NMR spectrum of polymer

![COSY NMR spectrum](image)

**Figure S3.** The COSY NMR spectrum of polymer in CDCl$_3$ at 300K (400 MHz).
7. Representative MALDI-TOF Mass Spectra

Figure S4. MALDI-TOF MS spectrum in reflector mode of the obtained 1a (([2]/[BnOH]/[DPP] = 20/1/1, reacted for 24 hours, conversion = 78%, $M_{n,NMR} = 4663$) prepared using a dry drop method with DHB as the matrix.
8. Water Contact Angle Tests

Figure S5. Water contact angle images for (a) blank, (b) mPEG-SH (5000 Da), (c) 1b (5364 Da, PDI = 1.01), (d) n-Hexadecanethiol.

9. Quartz crystal microbalance (QCM) assays

Figure S6. Adsorption profiles of BSA onto QCM Crystal surfaces modified by (a) nothing, (b) mPEG-SH (5000 Da), (c) 1b (5364 Da, PDI = 1.01), (d) n-Hexadecanethiol. The concentration of Bovine serum albumin Fraction V (BSA)
was /mL in PBS (PH = 7.40).

10. DSC Measurements

![DSC data of polymer 1a](image)

**Figure S7.** DSC data of 1a (15234 Da, PDI = 1.23). T_g = 73.3164°C. (10°C/min; -150 ~0°C; 2 cycles)

11. Degradation of Polymers.

The polyester (M_w 5423, 10 mg) was dissolved in methanol (5 mL). Sodium methoxide (50 mg of a 30 wt % solution in methanol) was added to the solution. The mixture was stirred for 3 h at room temperature, then neutralized with hydrochloric acid (2 M) and evaporated in vacuum. The residue was dispersed in THF, centrifuged and the Supernatant was subject to GPC analysis.

![GPC traces](image)

**Figure S8.** Representative GPC traces of polymer 1 (M_w 5423) before (black line) and after (red line) degradation.
12. Cell Adhesion Evaluation

Raw 264.7 cells were harvested from culture flasks and suspended in supplemented DMEM. The cells were counted using a haemocytometer and then diluted to a solution of 5×10^5 cells/mL. The SAMs and bare gold substrates were done in triplicates and placed in sterile Petri dishes. The substrates were then immersed in 4mL of DMEM. 1 mL Raw 264.7 cells suspension of 5 ×10^5 cells/mL was then added to each Petri dish to give a final cell suspension of 1 ×10^5 cells/mL in each Petri dish. The substrates were incubated for 24 h at 37°C in 5% CO₂. After incubation the substrates were rinsed in DMEM to ensure loosely bound cells were removed. The cells with Gold substrates stained with Calcien-AM, and then take photoes with Fluorescent microscope as a test for viability. The cells were counted also using the microscope.

13. Cell Cytotoxicity Assay

HFF cells were grown in 96-well plates at an initial density 5×10^3 cells per well for 24 h. Subsequently, the 150 and 200 μg/mL of PEG and polymer 1b were incubated for 12 h. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazoium bromide (MTT) reduction assay. After incubation, MTT (20 μL, 5 mg mL⁻¹) assay was added to each well for 4 h. DMSO (100 μL) was added to each well after removing media. Absorption at 490 nm was measured on a plate reader.
Figure S9. MTT assay of HFF cells treated with (a) PEG (8000 Da), (b) 1b (5364 Da, PDI = 1.01)

14. References

15. Appendix