Supplementary Materials for

"Non-swellable" hydrogel without mechanical hysteresis

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Materials and Methods

Dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), dichloromethane and ethyl acetate (Wako) were purified by conventional methods. Dulbecco's phosphate-buffered saline (D-PBS) (Wako) was used without purification. Potassium naphthalene was prepared as a THF solution (0.3 M), whose concentration was determined by titration. Pentaerythritol (>99%, Aldrich) was dried under vacuum. Ethyl glycidyl ether (EGE) (>98%, TCI) and methyl glycidyl ether (>97%, TCI) were purified by distillation from CaH_2 under atmospheric pressure. Ethyl bromoacetate (>97%, TCI), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (>98%, TCI), and *N*-hydroxysuccinimide (98%, Aldrich) were used without purification. Spectra/Por 7 (MWCO = 3500, Spectrum Laboratories) was used for dialysis. The molecular weight and functionality of the polymers were estimated by ^{1}H NMR (JEOL ECS 400) using tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvent. The polydispersity (M_w/M_n) was determined using a gel permeation chromatography system (TOSOH HLC-8220) that was equipped with two TSK gel columns (G4000HHR and G3000HHR). The columns were eluted with dimethylformamide containing lithium chloride (10 mM). The mass spectra were acquired on Ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) in linear positive ion mode.

Synthesis of Tetra-armed Polymer Units

The thermoresponsive unit, tetra-armed amine-terminated poly(ethyl glycidyl ether-*co*-methyl glycidyl ether) (Tetra-P(EGE_{190}-*co*-MGE_{47})-NH_2), was synthesized via the anionic polymerization of ethyl glycidyl ether and methyl glycidyl ether with the consecutive modifications of the end-groups (i.e., from a hydroxyl group to an amine group). The detailed procedures are described below:

Tetra-P(EGE_{190}-*co*-MGE_{47})-OH. Pentaerythritol (0.208 g, 1.53 mmol) was placed in a flask and dried under vacuum overnight. Under an argon atmosphere, a mixed solvent of DMSO and THF (v/v = 3/2) was slowly added, and the solution was stirred for 15 min. To obtain alkoxides, 0.3M potassium naphthalene (4.05 mL) was carefully injected into the solution. After the alkoxide formation, a mixture of ethyl glycidyl ether and methyl glycidyl ether (8:2 molar ratio) was injected into the flask at room temperature, and the solution was stirred at 60ºC. This process was repeated while the molecular weight was being monitored by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry until the desired molecular weight was obtained. After the monomers were completely consumed, the reaction was terminated by the addition of 5N HCl. The THF was evaporated, and the residue was dissolved in a large amount of ether, and subsequently washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The viscous liquid obtained was further purified by precipitation in cold hexane three times to remove the remaining naphthalene. Finally, the product was dried under vacuum overnight to yield Tetra-P(EGE_{190}-*co*-MGE_{47})-OH as a viscous yellowish liquid (Scheme S1). Mass spectrometry: M_w = 22,500. GPC: M_w/M_n = 1.02. ^{1}H NMR ($CDCl_3$): δ3.3-3.75 (m, 1595H, -C-CH_2-O-, EGE and MGE), δ1.17 (t, 546H, -O-CH_2-CH_3).

Tetra-P(EGE_{190}-*co*-MGE_{47})-COOH. In a two-neck flask, Tetra-P(EGE_{190}-*co*-MGE_{47})-OH (20 g, 0.889 mmol) was dissolved in THF (600 mL). To the solution, 0.3M

potassium naphthalene (123.2 mL) was added dropwise under an argon atmosphere. The solution remained dark green for 30 min, which indicated the complete deprotonation of the hydroxyl groups. Ethyl bromoacetate (7.635 mL, 73.7 mmol) was subsequently added at 0°C via syringe, and the solution turned yellow. The mixture was allowed to warm to room temperature, and was stirred for 24 h. After the obtained suspension was filtered, the THF and excess ethyl bromoacetate were subsequently removed *in vacuo*. The solution was treated with a mixture of 1N NaOH and 1,4-dioxane (v/v = 1/1) for 24 h at 80ºC. After the complete hydrolysis, the pH of the mixture was adjusted to 1. The solution was extracted into chloroform, and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*, and Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-COOH was recovered as a yellowish viscous liquid (Scheme S2). ^{1}H NMR ($CDCl_3$): δ4.27 (s, 8H, -O-C*H*$_2$-COOH), *δ*3.3-3.75 (m, 1595H, -C-C*H*$_2$-O-, EGE and MGE), δ1.17 (t, 546H, -O-CH$_2$C*H*$_3$).

Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-OSu. Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-COOH (17 g, 0.756 mmol) and *N*-hydroxysuccinimide (6.96 g, 60.48 mmol) were placed in a two-neck flask. Under an argon atmosphere, CH_2Cl_2 (510 mL) was added via a syringe. EDC (9.39 g, 60.48 mmol) in CH_2Cl_2 (340 mL) was slowly introduced at 0ºC. After the mixture was stirred overnight at room temperature, the solvent was removed under reduced pressure. The liquid obtained was dissolved in EtOAc, filtered and washed with saturated $NaHCO_3$ and then with water. Finally, the organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*, and Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-OSu was recovered as a viscous liquid (Scheme S3). ^{1}H NMR ($CDCl_3$): *δ*3.3-3.75 (m, 1595H, -O-C*H*$_2$-O-, EGE and MGE), *δ*2.84 (m, 16H, -O-CH$_2$-COO*Su*), *δ*1.17 (t, 546H, -O-CH$_2$C*H*$_3$).

Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-NH$_2$ (Thermoresponsive Unit). Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-OSu (10 g, 0.0444 mmol) was dissolved in CH_2Cl_2 (400 mL) in a flask while a mixture of ethylenediamine (58.86 mL, 889 mmol) and CH_2Cl_2 (100 mL) was prepared separately. With stirring, the polymer solution was injected dropwise via syringe into the flask that contained the ethylenediamine solution. The mixed solution was stirred for 3 hours. After the reaction, the solution was washed with water and brine before being dried over $MgSO_4$. Finally, dichloromethane was removed *in vacuo*, and the viscous yellow transparent polymer Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-NH$_2$ was recovered after lyophilization from benzene (Scheme S4). ^{1}H NMR ($CDCl_3$): *δ*4.14 (s, 7.5H*, -O-C*H*$_2$-CO-NH-), *δ*3.3-3.75(m, 1595H, -C-*CH*$_2$-O-, -CO-NH-C*H*$_2$- and EGE), *δ*2.82 (t, 7H*, -CH$_2$-*CH*$_2$-NH$_2$), *δ*1.17 (t, 546H, -O-CH$_2$C*H*$_3$). *The conversion ratio from the succinimidyl group to the amine group was estimated to be 0.88-0.94 by ^{1}H NMR.

Fabrication of Hydrogels

Hydrophilic tetra-armed succinimidyl- and amine-terminated poly(ethylene glycol) (M_w = 20,000) were prepared as previously reported (*6*). Tetra-armed amine-terminated poly(ethyl glycidyl ether-*co*-methyl glycidyl ether) was synthesized as a thermoresponsive polymer unit via the anionic polymerization of ethyl glycidyl ether and methyl glycidyl ether. We prepared hydrogels with different thermoresponsive segment ratios (*r*) by the injection of the aqueous solutions of hydrophilic and thermoresponsive polymer units via a dual syringe. Briefly, amine-terminated polymer units were dissolved in an aqueous phosphate buffer (pH 7.4, ionic strength = 25 mM) to obtain precursor solutions with the desired ratios of hydrophilic to thermoresponsive polymer units (0:10,

2:8, 4:6, 6:4, 8:2 and 10:0), while the succinimidyl-terminated polymer unit was separately prepared in an aqueous phosphate–citric buffer (pH 5.8, ionic strength = 25 mM). These pH and ionic strength values were chosen to control the gelation time. All the preparation steps were performed below 10°C so that the thermoresponsive polymer units remained soluble in the aqueous buffers. Then, the same volume of the succinimidyl and amine precursor solutions were simultaneously injected into the molds to obtain hydrogels. The polymer concentration was fixed at 6 mM, and the ratio of amine to succinimidyl end-groups was set to 1 throughout the gelation process.

Gelation Time Control

To control the gelation behavior, hydrogels were prepared with different buffer conditions. Amine-terminated polymer units were dissolved in an aqueous phosphate buffer (ionic strength = 0, 25, 50, 250 mM), while succinimidyl-terminated polymer units were separately prepared in an aqueous phosphate–citric buffer (ionic strength = 0, 25, 50, 250 mM), in which the thermoresponsive segment ratio (r) was fixed at r = 0.4. The buffered aqueous polymer solutions (6 mM) with the same ionic strength were injected into a vial at 10ºC. The gelation behavior was monitored by tilting the vial (Fig. S1).

Swelling Ratio in Hydrophilic and Thermoresponsive Segments

The swelling ratios in the hydrophilic (Q_h) and thermoresponsive (Q_t) segments were determined from the macroscopic volume change. First, the water content of the thermoresponsive segment (W_t) was gravimetrically determined from its polymer aqueous solution at 40°C ($W_t \approx 12\%$), which is an intrinsic value for each chemical compound. Assuming the invariance of W_t against the thermoresponsive segment ratio (r), we calculated the volume of the thermoresponsive segment at 40ºC (V_t). Consequently, the volume of the hydrophilic segment (V_h) was calculated by subtracting the total volume of the thermoresponsive units and the water existing nearby. Using the volume obtained above, Q_t is determined as $Q_t = V_t / V_{t\_0} \times 100$ and Q_h is similarly calculated as $Q_h = V_h / V_{h\_0} \times 100$ where V_{t_0} is the initial volume of the thermoresponsive segment and V_{h_0} is the initial volume of the hydrophilic segment, respectively.

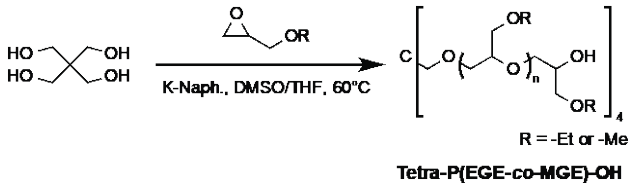
Mechanical Tests

All of the mechanical tests were performed immediately after the hydrogels were removed from D-PBS. The compression tests were carried out using a mechanical testing apparatus (INSTRON 3365, Instron) at a velocity of 0.75 mm/min. The elongation tests were performed with a mechanical testing apparatus (Autograph AG-X plus, SHIMADZU) at a crosshead speed of 60 mm/min.

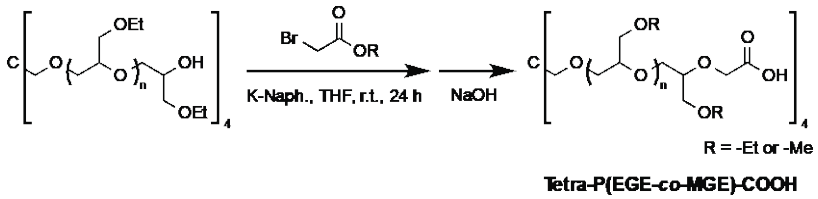
Degradation Test of Hydrogels equipped with Cleavable Linkage

Hydrogels that contain cleavable ester linkages were prepared according to the preparation steps used for the normal hydrogel assembly with a few modifications. At the step in which the aqueous solution of succinimidyl-terminated polymer unit was prepared, tetra-armed succinimidyl-terminated poly(ethylene glycol) with ester bonds (M_w = 20,000), which was previously reported (*6*), was quantitatively introduced. The ratio between tetra-armed succinimidyl-terminated poly(ethylene glycol)s “with and without” ester bonds was controlled simply by changing the mixing ratio of these polymer units.

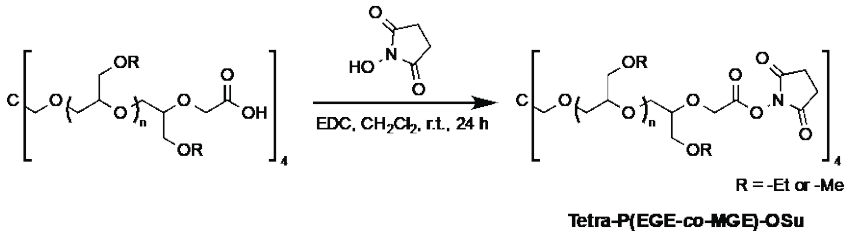
The ratio of the succinimidyl-terminated polymer units is defined as r_{deg}; for example, all the cross-linking points contain cleavable ester bonds at $r_{deg} = 1$. The thermoresponsive segment ratio (r) was fixed at $r = 0.4$ in this experiment. The degradation test was carried out on cylindrical hydrogel samples. Each sample was immersed in D-PBS at 37ºC, and the diameter change was recorded using an optical microscope (M165C, Leica). More than three samples were tested, and the observed diameters were arithmetically averaged.



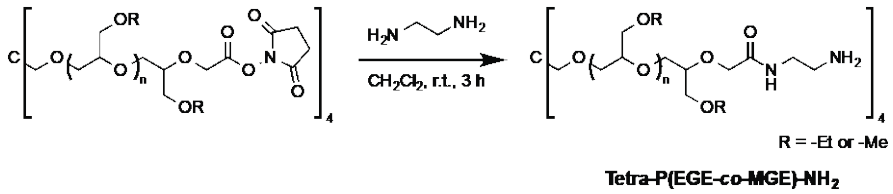
Scheme S1.
Synthetic route for Tetra-P(EGE$_{190}$-*co*-MGE$_{47}$)-OH



Scheme S2.
Synthetic route for Tetra-P(EGE_{190}-*co*-MGE_{47})-COOH



Scheme S3.
Synthetic route for Tetra-P(EGE_{190}-*co*-MGE_{47})-OSu



Scheme S4.

Synthetic route for Tetra-P(EGE_{190}-*co*-MGE_{47})-NH_2 (thermoresponsive unit)

Fig. S1.

Gelation behavior after the injection of aqueous buffered polymer solutions. The ionic strength values were controlled by properly preparing aqueous phosphate and phosphate–citric buffers, respectively. The polymer concentration was fixed at 6 mM.

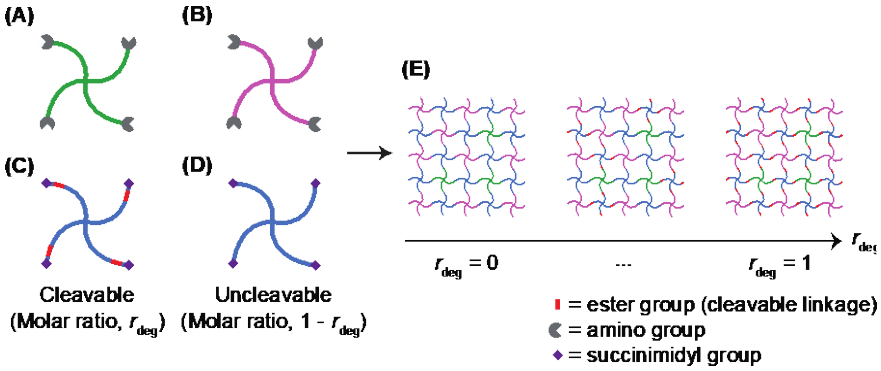


Fig. S2

Schematic of hydrogels equipped with cleavable linkage. (A) Tetra-armed poly(ethylene glycol) with ester bonds (red) and active ester end-groups (purple). **(B)** Tetra-armed poly(ethylene glycol) with active ester end-groups (purple). **(C)** Tetra-armed poly(ethyl glycidyl ether-*co*-methyl glycidyl ether) with amino end-groups (gray). **(D)** Tetra-armed poly(ethylene glycol) with amino end-groups (gray). **(E)** The polymer network structure of hydrogels containing cleavable ester linkages (red) that are quantitatively introduced as r_{deg} while the thermoresponsive segment ratio (r) is kept constant (r = 0.4). For example, when r_{deg} = 1, every polymer chain contains a cleavable linkage (left).

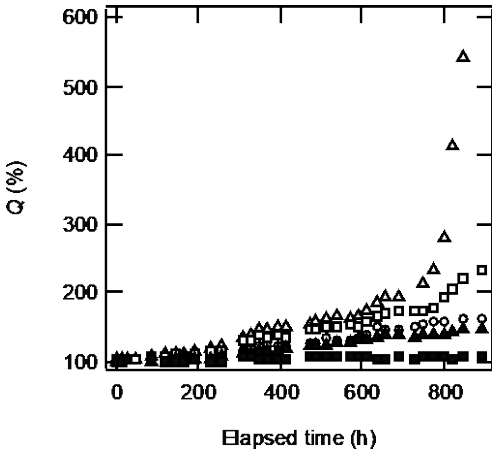


Fig. S3

Swelling ratio (*Q*) of hydrogels equipped with cleavable linkages as a function of time in D-PBS at 37ºC. *Q* is defined as V_t / V_0 × 100, where V_t is the volume of the samples at the time of observation and V_0 is the initial volume of the samples. The symbols represent the cleavable unit ratio (r_{deg}); r_{deg} = 0 (■), 0.7 (▲), 0.8 (○), 0.9 (□) and 1 (△). The thermoresponsive segment ratio (*r*) was kept constant (*r* = 0.4). For the sample with r_{deg} = 1, complete dissolution occurred within 850 hours.