# **Electronic Supplementary Information (ESI)**

for

## Biodegradable injectable polymer systems exhibiting longer and

# controllable duration time of the gel state

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#### Text S1. Synthesis of tri-PCG

We synthesized PCGA-b-PEG-b-PCGA triblock copolymer (tri-PCG) as a thermogelling polymer according to the method reported previously<sup>S1-3)</sup>. PCGA-*b*-PEG-*b*-PCGA triblock copolymers were synthesized by ring-opening copolymerization of CL and GL in the presence of PEG<sub>1500</sub> as a macroinitiator and Sn(Oct)<sub>2</sub> as a catalyst at 160°C for 12 h. Typically, PEG<sub>1500</sub> (16.0 g, 10.4 mmol) in 100 ml flask was dried under vacuum at 140°C for 3 h. After cooling to r.t., CL (34.4 g, 302 mmol), GL (6.04 g, 52.1 mmol) and Sn(Oct)<sub>2</sub> (151 mg, 372 µmol) [molar ratio of CL to GL (CL/GL) was 5.8] were added to the flask and dried under vaccum at r.t. for 12 h. Polymerization was then carried out at 160°C for 12 h, the product was purified by reprecipitation using chloroform (100 ml) as a good solvent and diethylether (1000 ml) as a poor solvent to give white solid of tri-PCG (tri-PCG-1). Similar procedures were carried out by changing feeding ratios of CL/GL/PEG to give tri-PCG-1 and tri-PCG-2 with different chain length and CL/GA ratios.  $M_{\rm n}$  and the average degrees of polymerization for CL and GA units in a PCGA segment were estimated by <sup>1</sup>H-NMR (solvent: CDCl<sub>3</sub>).  $M_{\rm w}$  and the polydipersity index  $(M_w/M_n)$  were estimated by SEC. Average continuous sequence lengths of caproyl units and glycolyl units ( $L_c$  and  $L_g$ ) in a PCGA segment were also estimated from <sup>1</sup>H-NMR spectra according to the literature <sup>S1-3)</sup>. The results are summarized in Table S1.

#### **References for supporting information**

S1) P. Dobrzynski, S. Li, J. Kasperczyk, M. Bero, F. Gasc, M. Vert, Structure-Property Relationships of Copolymers Obtained by Ring-Opening Polymerization of Glycolide and ε-Caprolactone. Part 1. Synthesis and Characterization. Biomacromolecules 6 (2005) 483-488.

S2) J. W. Pack, S. H. Kim, I-W. Cho, S. Y. Park, Y. H. Kim, Microstructure Analysis and Thermal Property of Copolymers Made of Glycolide and ε-Caprolactone by Stannous Octoate. J. Polym. Sci. A. Polym. Chem. 40 (2002) 544-554.

S3)Y. Yoshida, A. Takahashi, A. Kuzuya, Y. Ohya, Instant preparation of a biodegradable injectable polymer formulation exhibiting a temperature-responsive solgel transition, Polym. J. 46 (2014) 632-635.



Scheme S1. Synthesis of PCGA-b-PEG-b-PCGA triblock copolymer (tri-PCG).

Table S1. Characterization of PCGA-b-PEG-b-PCGA triblock copolymers (tri-PCGs)

Code	DP of CL <sup>a)</sup>	DP of GA <sup>b)</sup>	CL/GA <sup>c)</sup>	$L_{\rm C}^{\rm d)}$	$L_{\rm G}^{\rm d}$	$M_{\rm n}$ (Da) <sup>f)</sup>	$M_{ m w}/M_{ m n}{}^{ m g)}$
tri-PCG-1	14	4.2	3.4	3.6	1.1	5,300	1.4
tri-PCG-2	9.4	2.6	3.7	3.8	1.0	4,000	1.4

a) Degree of polymerization of ε-caprolactone unit in a PCGA segment calculated from the methylene peaks in <sup>1</sup>H-NMR.

b) Degree of polymerization of glycolic acid unit in a PCGA segment calculated by <sup>1</sup>H-NMR.

c) Molar ratio of CL/GA in a PCGA segment estimated by  $^{1}$ H-NMR.

d) Average continuous sequence lengths of caproyl units and glycolyl units,  $L_c$  and  $L_g$ , were calculated by the following equations (ref. S1-S2) using data in <sup>1</sup>H-NMR.

e) Estimated by a differential scanning calorimeter.

f) Estimated by <sup>1</sup>H-NMR (solvent: CDCl<sub>3</sub>).

g) Estimated by size-exclusion chromatography (eluent: DMF, standard: PEG).

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Code	$M_n$ (Da) <sup>b)</sup>	$M_{w}$ (Da) <sup>c)</sup>	$M_{\rm w}/M_{\rm n}$ °)	Degree of substitution (%) $^{d)}$
Tri-PCG-Acryl	4,100	4,200	1.3	92
			1	

Table S2. Characterization of tri-PCG-Acryl

a) Number-average molecular weight estimated from <sup>1</sup>H-NMR spectra.

b) Estimated by size-exclusion chromatography (eluent: DMF, standard: PEG).

c) Degree of substitution of acryloyl group calculated by <sup>1</sup>H-NMR.



Figure S1. <sup>1</sup>H-NMR spectra for (a) tri-PCG-2 and (b) tri-PCG-Acryl in CDCl<sub>3</sub>.



**Figure S2.** SEC elution curves for tri-PCG-2 (dotted red line) and tri-PCG-Acryl (solid blue line) (eluent: DMF, standard: PEG).



Figure S3. Photographs of (a) tri-PCG-1 and (b) tri-PCG-Acryl in dry state at r.t.

Text S2. Sol-to-gel transition of tri-PCG, tri-PCG-Acryl, and their mixtures.

The temperature-responsive sol-to-gel transition behavior of the copolymers, tri-PCG-1 and tri-PCG-Acryl, and their mixtures (tri-PCG-1/tri-PCG-Acryl = 1/1, 2/1 and 5/1 (w/w)) was investigated in PBS at concentration range from 15-25 wt% by a test-tube inverting method. These all samples were prepared by heating dissolution. The results are shown in Table S3.

Pure tri-PCG-1 solution (S(P1)), and the 2/1 and 5/1 mixture solution of tri-PCG/tri-PCG-Acryl exhibited temperature-responsive sol-to-gel transitions at 15-25 wt%. The 1/1 mixtures of tri-PCG/tri-PCG-Acryl and pure tri-PCG-Acryl solution (S(PA)) showed gelation at 20-25 wt% and only 25 wt%, respectively. These results indicate that mixing with tri-PCG-Acryl (less than 50 wt%) did not have a great influence on the sol-to-gel transition behavior of the tri-PCG-1 solution.

Sample	Total polymer	sol to gel	gel to sol(syneresis)
	concentration	transition	transition
	(wt%)	(°C)	(°C)
	15	38	41
tri-PCG	20	34	43
	25	33	47
	15	N.D. <sup>a)</sup>	34 <sup>b)</sup>
tri-PCG-Acryl	20	N.D. <sup>a)</sup>	37 <sup>b)</sup>
	25	32	50
	15	N.D. <sup>a)</sup>	37 <sup>b)</sup>
	20	35	44
1/1	25	33	48
	15	37	42
tm-PCG-Acryl/m-PCG	20	35	44
1/2	25	33	48
	15	37	42
	20	35	44
1/3	25	33	50

**Table S3.** Gel to sol and gel to sol (syneresis) transition for tri-PCG, tri-PCG-Acryl, and their mixture in PBS.

a) Transition was not detected.

b) Transition from sol state to syneresis.

#### Text S3. Confirmation of incorporation of DPMP in tri-PCG micelle

In Figure S4, <sup>1</sup>H-NMR spectrum of DPMP saturated in D<sub>2</sub>O are shown. Using the spectrum, the solubility of DPMP was calculated from the internal reference (methanol) to be 193.4  $\mu$ g/mL. The results mean the solubility of DPMP is not high, but DPMP can actually be detected in D<sub>2</sub>O by <sup>1</sup>H-NMR. Figure S5 shows <sup>1</sup>H-NMR spectrum of tri-PCG micelle containing DPMP in D<sub>2</sub>O. The peaks of DPMP were not detected, because DPMP was in solid-like state by incorporating into the micelle core. On the other hand, the aqueous solution of tri-PCG micelle containing DPMP was freeze-dried, and re-dissolved in DMSO-*d*6. The <sup>1</sup>H-NMR of the obtained solution was shown in Figure 6B. The peak assigned to DPMP was clearly observed around 2.65 ppm. Such peak was not observed in <sup>1</sup>H-NMR of tri-PCG in DMSO-*d*6 (Figure 6C). These all results mean DPMP was existed and incorporated in the tri-PCG micelle in aqueous solution.



**Figure S4.** <sup>1</sup>H-NMR spectrum of saturated DPMP in  $D_2O$  in the presence of 0.1% methanol (v/v) as internal reference.



**Figure S5.** <sup>1</sup>H-NMR spectrum of tri-PCG micelle containing DPMP in D<sub>2</sub>O.



**Figure S6.** <sup>1</sup>H-NMR spectra for (A) DPMP in DMSO- $d_6$ , (B) tri-PCG micelle containing DPMP after freeze-drying and re-dissolved in DMSO- $d_6$ , (C) tri-PCG in DMSO- $d_6$ .



Figure S7. Photographs of (a)  $S(P1/D+PA_{40})$ , (b)  $S(P1/D+PA_{36})$ , (c)  $S(P1/D+PA_{33})$ , (d)  $S(P1/D+PA_{17})$ , (e) S(P1), (f) S(P1/D) and (g) S(PA) after preparation at 25 °C, subsequent heating at 37°C for 1 min, and further cooling at 4°C for 1 min. Total copolymer concentration = 25 wt%.



**Figure S8.** Storage (*G*', closed symbols) and loss moduli (*G*'', open symbols) of *S*(P1) ( $\bullet$ ,  $\bigcirc$ ), *S*(P1/D) ( $\blacksquare$ ,  $\Box$ ), *S*(PA) ( $\blacktriangle$ ,  $\triangle$ ) as a function of temperature. Total copolymer concentration = 25wt%



**Figure S9.** Time course of storage (*G*<sup>'</sup>, closed symbols) and loss moduli (*G*<sup>''</sup>, open symbols) for (a)  $S(P1/D+PA_{40})$ , (b)  $S(P1/D+PA_{36})$ , and (c)  $S(P1/D+PA_{17})$  after heating to 37°C and subsequent cooling to 25°C. Total copolymer concentration = 25wt%. Temperature change schedule was shown in the bottom of each Figure.



**Figure S10.** IR spectra for (A) *S*(P1/D+PA<sub>40</sub>), (B) *S*(P1/D) (tri-PCG containing DPMP), and (C) *S*(PA) (tri-PCG-Acryl) after gelation and freeze-drying by KBr method.



Figure S11. Differential IR spectra  $S(P1/D+PA_{40}) - [0.6 \times S(P1/D) + 0.4 \times S(PA)]$ .

### Text S4. IR spectra measurements.

We measured IR spectra for the samples after freeze-drying using a FT-IR spectrometer (Perkin-Elmer 1600). Figure S10 shows the IR spectra for  $S(P1/D+PA_{40})$ , S(P1/D), and S(PA) after gelation and freeze drying. However, these spectra are quite similar, and any obvious differences were not detected. Then, we calculated the differential IR spectra  $S(P1/D+PA_{40}) - [0.6 \times S(P1/D) + 0.4 \times S(PA)]$  (Figure S11). In the differential spectrum, slight absorbance around 2900 cm<sup>-1</sup> which can be assigned to C-S-C bonds was observed. The result suggests the formation of new S-C bonds by thiol-ene reaction.



**Figure S12.** Time course of storage moduli of previously reported system using NH<sub>2</sub>/OSu coupling after heating to 37 °C and further maintaining at 37 °C. [ref.35]



**Figure S13.** Degradation profiles of tri-PCG-1 hydrogel (*S*(**P1**)) soaked in PBS at 37 °C.

30days after s.c. implantation



Figure S14. Photograph of  $S(P1/D+PA_{40})$  30 days after s.c. implantation and soaking in acetone.