Electronic Supplementary Information (ESI)

Synthesis of degradable double network gels using a hydrolysable cross-linker

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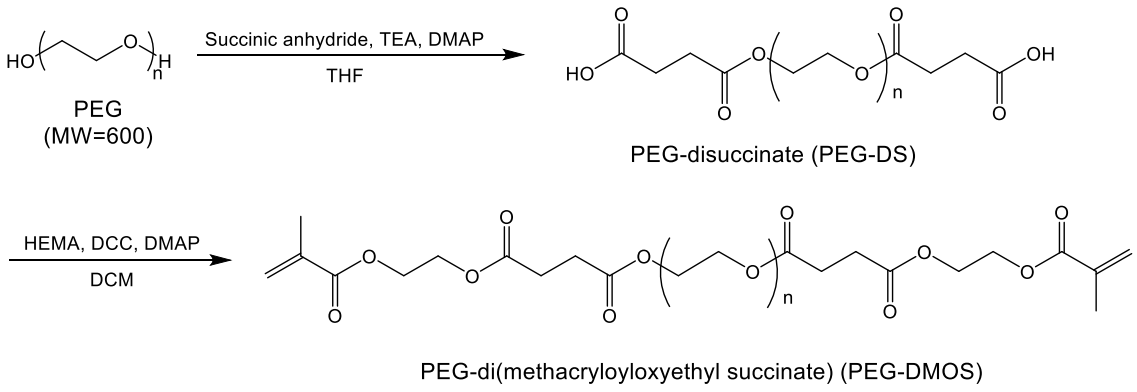
Experimental

1. Materials

Poly(ethylene glycol) (PEG) (molecular weight (MW) = 600 g/mol), succinic anhydride (SA), 2-aminoethanthiol, *N,N*-dimethylacrylamide (DMAAm), acrylamido-2-methylpropane sulfonic acid (AMPS), 2-oxoglutaric acid, and other chemicals for synthesis and organic solvents were purchased from FUJIFILM Wako Pure Chemical Ind., Ltd. (Osaka, Japan). PEG-diacrylate (PEG-DA) (MW = 700 g/mol) was purchased from Sigma-Aldrich (St. Louis, USA). 2-Hydroxyethylmethacrylate (HEMA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Branched PEG with 4 arms (4-arm PEG) (MW = 5,000 g/mol) and 4-arm PEG derivative with terminal thiol groups (SUNBRIGHT PTE100SH) (4-arm PEG_{10K}-SH) (MW=10,000 g/mol) was obtained from NOF Co. (Tokyo, Japan). Monomers and organic solvents were purified by usual distillation. Water was purified using Millipore Elix UV3 direct-Q UV (Merck, Darmstadt, Germany) and used after N_2 bubbling.

2. Synthesis of PEG-DMOS and PDMAAm/PEG-DMOS gel

The synthesis of PEG-di(methacryloyloxyethyl succinate) (PEG-DMOS) was carried out according to **Scheme S1**, as follows.



Scheme S1. Synthesis of PEG-DMOS.

2.1. PEG-disuccinate (PEG-DS)

SA (3.42g, 33.2mmol) was dissolved in 10 mL of tetrahydrofuran (THF) under N_2 atmosphere at 0°C. PEG (MW = 600 g/mol, 10.02g, 16.7mmol) solution in 10 mL of THF containing 2.0 mL of TEA was added to the SA solution under N_2 atmosphere at 0°C and stirred at 0°C for 2 h and 25°C for 24h. After evaporation, 20 mL of 1M HCl aq. was added. After extracting with diethyl ether and washing, the objective PEG-disuccinate (PEG-DS) was obtained. The product was characterized by ^{1}H-NMR (**Figure S1**). Yield: 12.72g (95.5%).

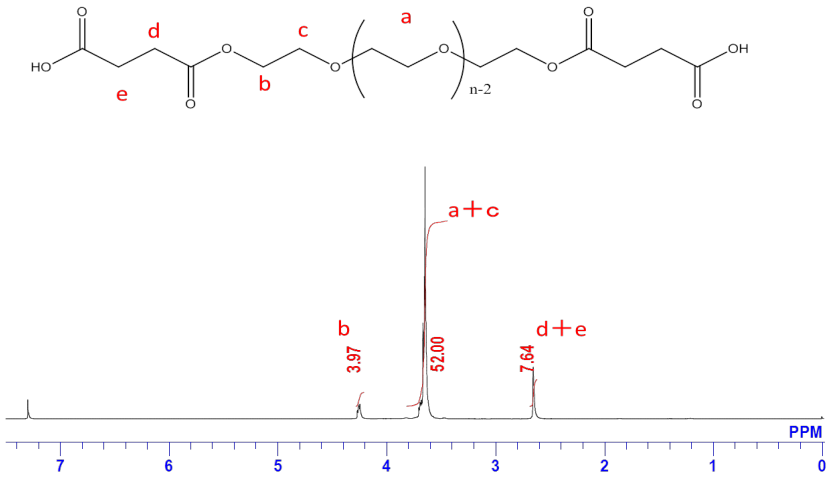


Figure S1. ^{1}H-NMR spectrum for PEG-disuccinate (PEG-DS) (Solvent: $CDCl_3$).

2.2. PEG-di(methacryloyloxyethyl succinate) (PEG-DMOS)

PEG-DS (0.4 g, 0.5 mmol), dicyclohexylcarbodiimide (DCC) (0.32 g, 1.5 mmol) and dimethylamino-pyridine (DMAP) (8.7 mg, 0.071 mmol) were put in a flask and dissolved in 2.0 mL of dichloromethane (DCM) under N_2 atmosphere. Then HEMA (0.2 g, 1.5 mmol) was added to the flask and stirred for 24 h. After removing dicyclohexylurea (DCUrea, a byproduct) by filtration, the product was purified by reprecipitation using chloroform and hexane as good and poor solvents, respectively. Yield: 412 mg (81%). The degree of introduction of HEMA unit was estimated to be 82% by ^{1}H-NMR **(Figure S2)**.

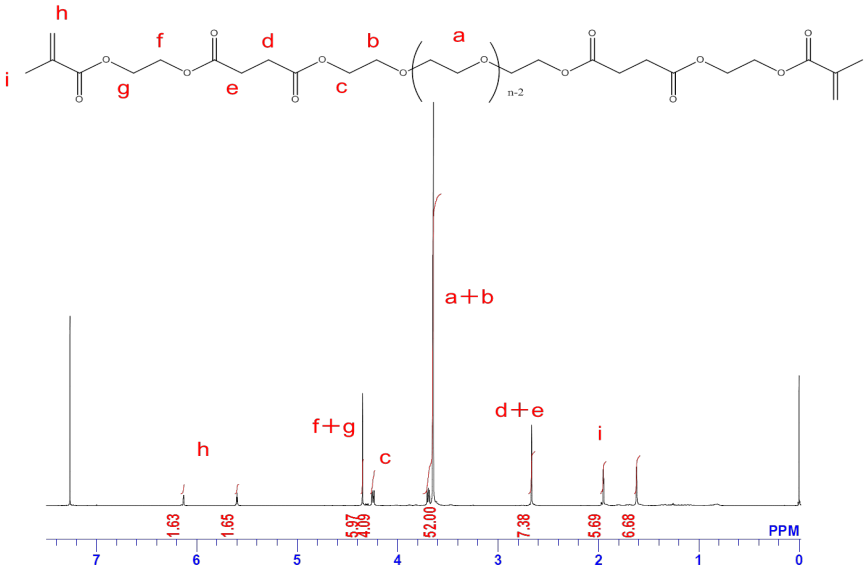
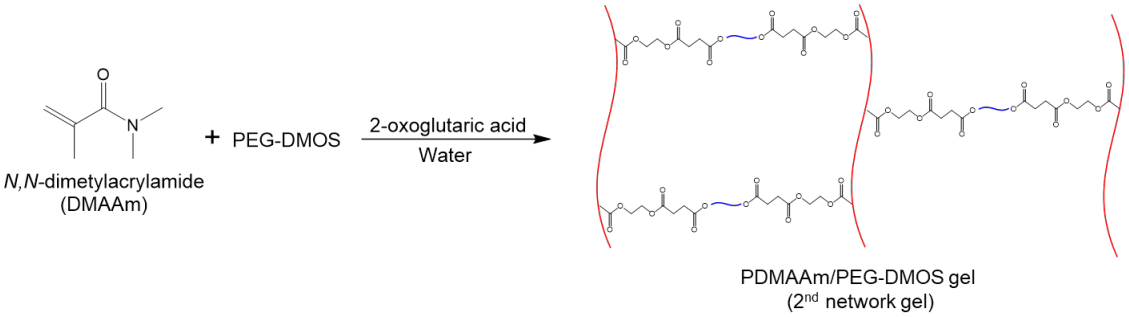


Figure S2. ^{1}H-NMR spectrum for PEG-di(methacryloyloxyethyl succinate) (PEG-DMOS) (Solvent: $CDCl_3$).



Scheme S2. Synthesis of PDMAAm/PEG-DMOS gel (2nd NW gel).

2.3. Synthesis of PDMAAm/PEG-DMOS gel (2nd network (NW) gel)

DMAAm (200 mg, 2.0 mmol) and PEG-DMOS (2.2 mg, 0.2 μmol) or PEG-DA (1.4 mg, 0.2 μmol) were dissolved in water (0.8 mL), and 2-oxoglutaric acid (0.3 mg, 2.0×10^{-3} mol) was added to the solution. The solution was put in a mold (10 × 10 × 3 mm) of glass plates and silicone rubber (**Figure S3**), and UV light (λ= 365 nm) (AS ONE SLUV-8, Osaka, Japan) was irradiated for 6 h. The PDMAAm/PEG-DMOS gel and PDMAAm/PEG-DA gel were washed with water before use. Transparent gels were obtained. The photographs of the obtained gels before and after hydrolysis are shown in **Figure S4**.

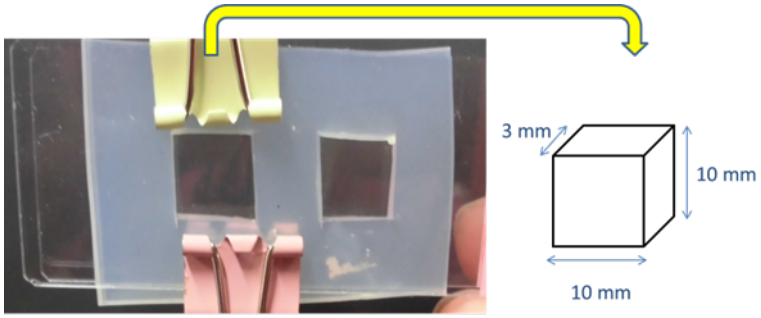


Figure S3. Photograph of the mold for gel preparation.

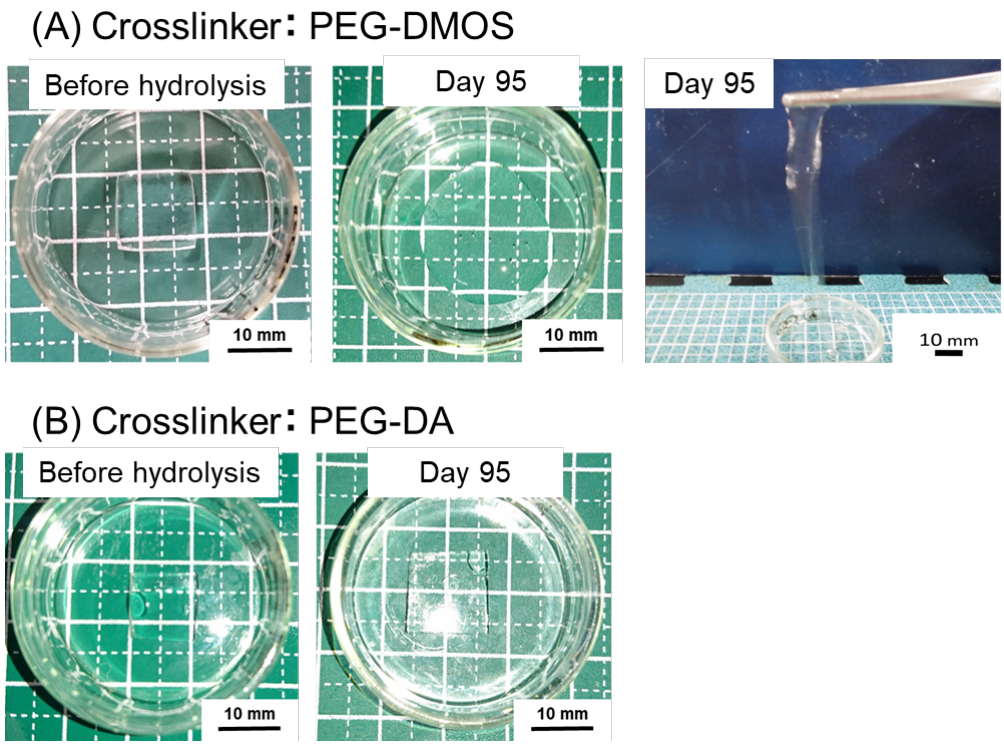
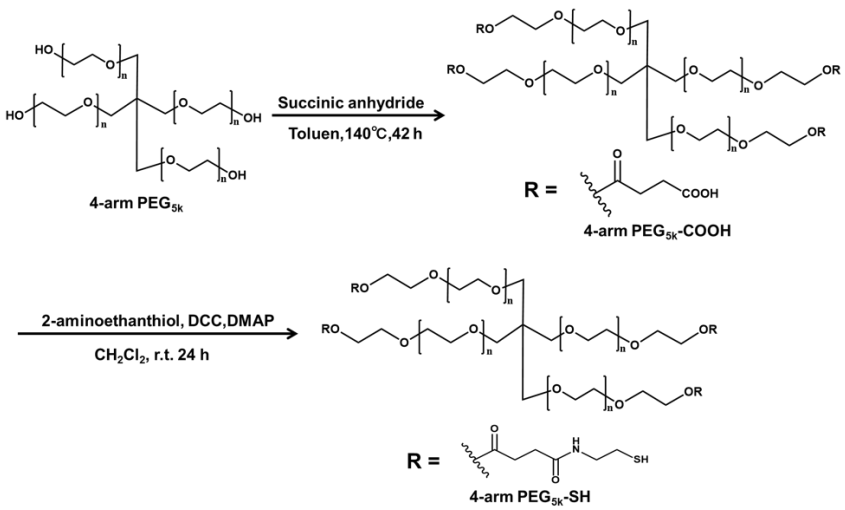


Figure S4. Photographs of (A) PDMAAm/PEG-DMOS gel and PDMAAm/PEG-DA gel before and after 95 days hydrolysis in PBS (pH =7.4) at 37°C.

3. Synthesis of 4-arm PEG/PEG-DA gel (PEG gel, 1st NW gel)

3.1. Synthesis of 4-arm PEG$_{5k}$-SH

4-Arm PEG$_{10k}$-SH (MW = 10,000 g/mol) was obtained from NOF Co. and used without further purification. 4-Arm PEG$_{5k}$-SH (MW = 5,000 g/mol) was synthesized from 4-arm PEG$_{5k}$ as follows (**Scheme S3**). 4-arm PEG$_{5k}$ (100 mg, 0.02 mmol) and SA (40.2 mg, 0.4 mmol) were dissolved in toluene, and refluxed at 140°C for 42 h. After removing unreacted succinic anhydride by suction filtration, the product was purified by reprecipitation using chloroform and diethyl ether/methanol (9/1) as good and poor solvents, respectively. The purity and degree of introduction of carboxylic acid groups were confirmed by ^{1}H-NMR (**Figure S5**). Yield 102 mg (94%). The degree of introduction of COOH groups per OH was 95.6%. The obtained 4-arm PEG$_{5k}$-COOH (100 mg, 0.02 mmol) was dissolved in anhydrous DCM. 2-Aminoethanthiol (9.3 mg, 0.12 mmol), DCC (24.8 mg, 0.12 mmol), and DMAP (4.9 mg, 0.04 mmol) were put in another flask and dissolved in anhydrous DCM. The obtained 2-Aminoethanthiol/DCC/DMAP solution was added dropwise to the 4-arm PEG$_{5k}$-COOH solution at 0°C and further stirred at 25°C for 24h. After removal of DCUrea, the product was purified by reprecipitation 3 times using chloroform and n-hexane/methanol (8/2) as good and poor solvents, respectively. The obtained 4-arm PEG$_{5k}$-SH was characterized by ^{1}H-NMR **(Figure S6)**. Yield: 103 mg (97%). The degree of introduction of thiol groups was over 99% per COOH group.



Scheme S3. Synthesis of 4-arm PEG_{5k}-SH.

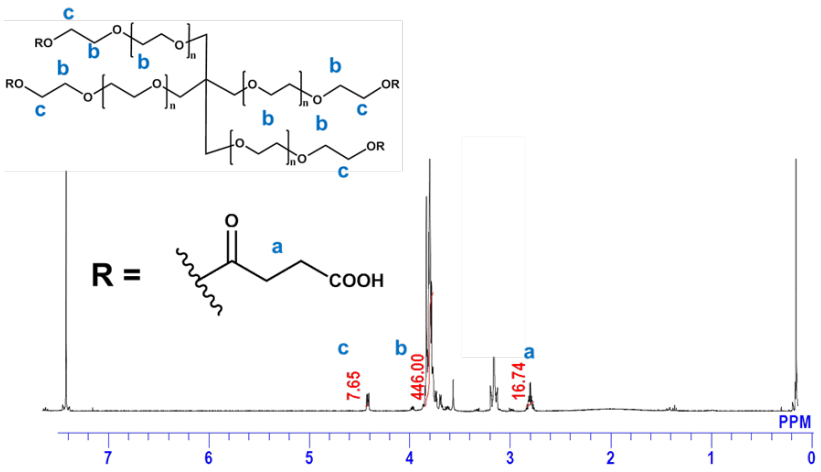


Figure S5. ^{1}H-NMR spectrum of 4-arm PEG_{5k}-COOH (Solvent: $CDCl_3$).

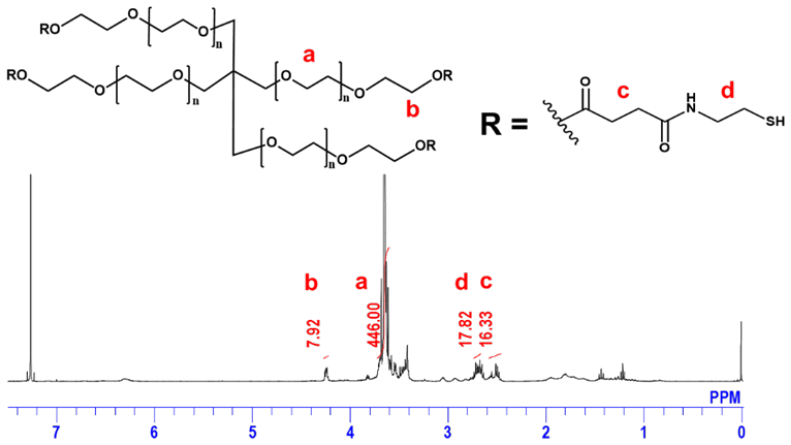


Figure S6. ^{1}H-NMR spectrum of 4-arm PEG_{5k}-SH (Solvent: $CDCl_3$).

3.2. Synthesis of PEG gel (1st NW)

4-Arm PEG/PEG-DA gels, **PEG5k gel** and **PEG10k gel**, (for 1st NW) were synthesized by thiol-ene reaction of 4-arm PEG_{5k}-SH and 4-Arm PEG_{10k}-SH with PEG-DA (MW = 700), respectively (**Scheme 1**, in the main text). 4-Arm PEG_{5k}-SH (or 4-Arm PEG_{10k}-SH) and PEG-DA were separately dissolved in phosphate buffer (pH = 7.4). The concentrations of the macromonomers were varied from 5 to 20 wt%, where the feeding ratio of SH group to acrylate group was 1:1. After mixing these solutions with stirring, the mixed solution was poured into a mold shown in **Figure S3.** The mold was kept at 37°C for 24 h. The obtained gel was immersed in water for 2 days (the water was replaced every 12 h) to remove salts and unreacted compounds, resulting equilibrium swelling. The photographs of the examples of the obtained **PEG5k gel** and **PEG10k gel** are shown in **Figure S7**. Transparent gels were obtained.

4. Preparation of St-DN gel

4.1. Polymerization of AMPS in PEG gel

Preparation of stent containing double NW gel (**St-DN gel**) was prepared basically according to the reference[1,2] as follows (**Scheme 1**, main text). The polymerization of AMPS in **PEG gel** to introduce PAMPS as a stent was carried out. The prepared **PEG_{5k} gel** or **PEG_{10k} gel** was immersed in 5 mL of an aqueous solution containing 273-2071 mg of AMPS (concentration = 0.2-2.0 M). The pH of the solution was adjusted to pH = 5-6 using 1M NaOH aq. After soaking at 4°C for 24 h, the swelled gel was sandwiched two glass plates and irradiated with UV light (λ= 365 nm) (AS ONE SLUV-8, Osaka, Japan) for 6 h under N_2 atmosphere. The obtained **St-PEG_{5k} gel** and **St-PEG_{10k} gel** containing PAMPS were immersed in water to wash, resulting equilibrium swelling. Transparent gels were obtained. **Figure S7** shows the typical examples for (A) **PEG_{5k} gels** and **St-PEG_{5k} gels** and (B) **PEG_{10k} gels** and **St-PEG_{10k} gels**, where AMPS concentration was 1.0 M immersed in water. Higher swelling for **St-PEG gels** were observed.

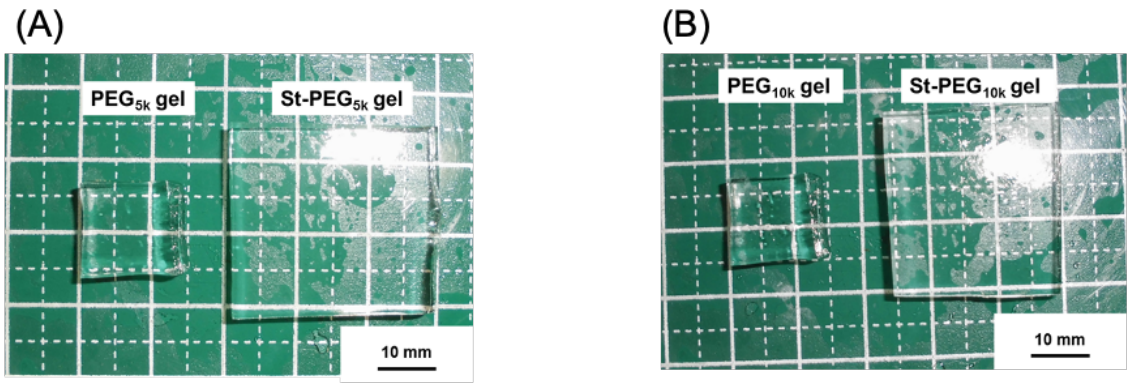


Figure S7. Photographs of (A) **PEG_{5k} gel** and **St-PEG_{5k} gel**, (B) **PEG_{10k} gel** and **St-PEG_{10k} gel**. AMPS concentration = 1.0 M.

4.2. Preparation of St-DN gel

A predetermined amount of DMAAm, PEG-DMOS and 2-oxoglutaric acid were dissolved in water (concentration of DMAAm = 0.5 – 10 M, the amount of PEG-DMOS = 0.001 – 0.5 mol% for DMAAm. The feeding ratio of 2-oxoglutaric acid/DMAAm = 1/1000). The **St-PEG$_{5k}$ gel** and **St-PEG$_{10k}$ gel** prepared above were immersed in the solution. After soaking at 4°C for 24 h, the swelled gel was sandwiched two glass plates and irradiated with UV light (λ= 365 nm) (AS ONE SLUV-8, Osaka, Japan) for 6h under N_2 atmosphere to give **St-DN$_{5k}$ gel** and **St-DN$_{10k}$ gel**, respectively. Transparent gels were obtained. The obtained gels were immersed in water to wash, resulting equilibrium swelling. **Figure S8** shows the typical examples for (A) **St-DN$_{5k}$ gel** and (B) **St-DN$_{10k}$ gel**.

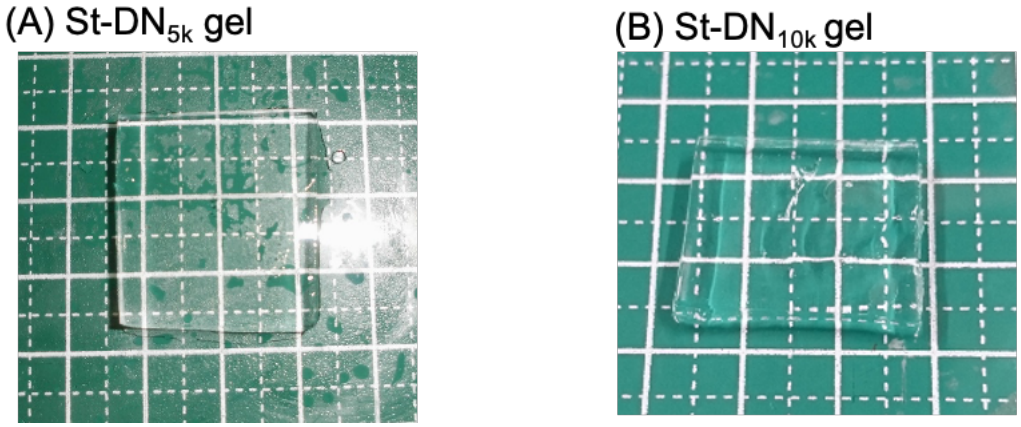


Figure S8. Photograph of the examples for **St-DN gels**. (A) **St-DN$_{5k}$ gel** (concentration of DMAAm = 2.0 M, crosslinker = 0.01 mol% for monomer) and (B) **St-DN$_{10k}$ gel** (concentration of DMAAm = 7.5 M, crosslinker = 0.1 mol% for monomer). The scale of the square background = 1 cm.

5. Degradation test

Degradation tests were carried out by measuring the gel weight (swelling) under physiological conditions (phosphate-buffered saline (PBS), pH = 7.4). The gel was immersed in PBS (pH =7.4) at 37°C. At the predetermined time, the weight of a swelled gel was measured. The swelling ratio was calculated by the following equation.

Swelling ratio = swelled gel weight at measurement (g) / dry gel weight (initial) (g)

6. Measurements

^{1}H-Nucleic magnetic resonance (NMR) spectra were obtained using a GSX-400 spectrometer (JEOL, Tokyo, Japan). The mechanical strength of the gels was characterized by compressive stress-strain measurements, which were performed on water-swollen gels using an Autograph AGS-J series

instrument (Shimadzu, Kyoto, Japan) at room temperature. The cylindrical gel sample (10 mm diameter. 3 mm thick) was set on the lower plate and compressed by the upper plate, connected to a load cell at a strain rate of 10%/min. Strain was defined as the thickness change divided by the thickness of the free-standing state. Tensile testing was also carried out on strip specimens using the same instruments with a 30 mm/min crosshead speed. The strip specimens (5 × 10 × 3 mm thick) were cut out from the gel.

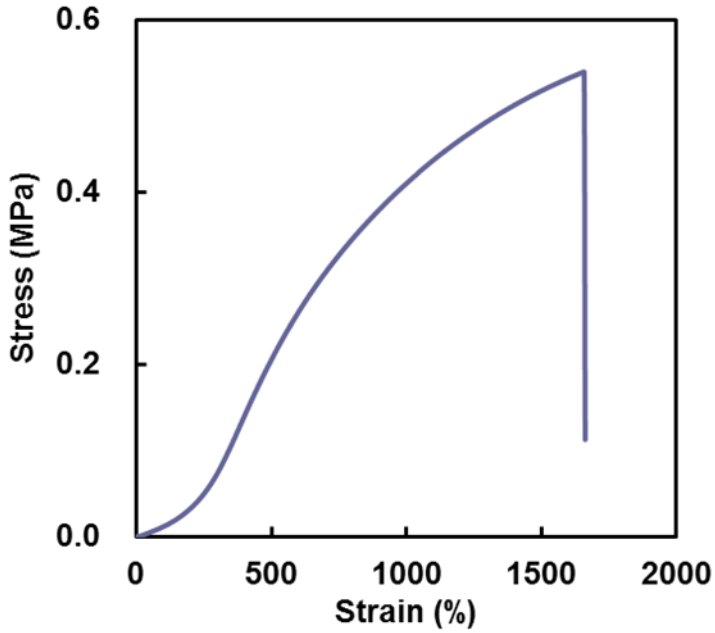


Figure S9. Results of tensile strength test for **St-DN$_{5k}$ gel** (concentration of DMAAm = 2.0 M, crosslinker = 0.01 mol% for monomer).

Table S1 Comparison in mechanical strength for the DN gels reported

DN gels	Compressive test		Tensile test		
Components expressed as 1st NM(Stent)/2nd NW	Fracture strength (MPa)	Fracture Strain (%)	Fracture strength (MPa)	Elongation to break (%)	Ref. No. (in main text)
St-PEG5k DN gel PEG(PAMPS)/PDMAAm	**20**	**99**	**0.5**	**1600**	**This study**
DN gel PAMPS(-)/PAAm	17.2	90	1	1500	10,11
St-DN gel PHEA(PAMPS)/PAAm			1	1200	17
St-DN gel PEG(PAMPS)/PAAm (non-degradable)	>40	>95	2	2000	18,19
DN gel BC(-)/Gelatin	3.8	40	2.7 (Fiber axis)	18 (Fiber axis)	39
PEA-UPyA/PEA-FMA (Dynamic cross-linking with UPy)			0.5	180	15
SPEB/HTPB (Dynamic cross-linking with UPy group)			8	70	16

PEG: poly(ethylene glycol)
PAMPS: poly(2-acrylamido-2-methylpropane sulfonic acid)
PDMAAm: polydimethylacrylamide
TPEG: tetra-PEG
PAAm: polyacrylamide
BC: bacterial cellulose
PEA: poly(ethyl acrylate)
UPy: ureido-pyrimidine-acrylate
FMA: furfuryl methacrylate
SPEB: poly(ethylene-co-1-butene) functionalized UPy group
HTPB: hydroxyterminated polybutadiene

References

1) T. Nakajima, H. Sato, Y. Zhao, S. Kawahara, T. Kurokawa, K. Sugahara, J. P. Gong, *Adv. Funct. Mater*. 2012, **22**, 4426–4432.
2) T. Nakajima, Y. Fukuda, T. Kurokawa, T. Sakai, U.-I. Chung, J. P. Gong, *ACS Macro Lett.,* **2013, 2**, 518–521.