[Supporting Information]

Oligo(amidoamine)s hydrogels with tunable gel properties

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Experimental Section

Materials:

N,N'-Methylene diacrylamide (MDA) 1,6-diaminohexane (DAH), 1,8-diaminooctane (DAO), 1,10-diaminodecane (DAD), and 4,4'-trimethylene dipiperidine (TMDP), methanol (MeOH), chloroform ($CHCl_3$), acryloyl chloride (AC), triethylamine (TEA), were purchased from Aldrich Chemical Co. Acetone was used as received from Samchun (Korea). All other reagents were of analytical grade and used without further purification.

Synthesis of 1,10-decylene diacrylamide (DDA), 1,8-octylene diacrylamide (ODA) and 1,6-hexylene diacrylamide (HDA): DDA, ODA and HDA were synthesized in previous report.[1] Their chemical structures were confirmed by ^{1}H-NMR in DMSO.

^{1}H-NMR of DDA (500 MHz, DMSO) δ (ppm): ^{1}H-NMR of ODA (500 MHz, DMSO) δ (ppm): 6.17-6.22 (q, 2H), 6.03-6.07 (q, 2H), 5.53-5.56 (q, 2H), 3.08-3.12 (m, 4H), 1.4-1.43 (t, 4H), 1.24-1.31 (s, 12H).

^{1}H-NMR of ODA (500 MHz, DMSO) δ (ppm): 6.17-6.22 (q, 2H), 6.03-6.07 (q, 2H), 5.53-5.56 (q, 2H), 3.08-3.12 (m, 4H), 1.4-1.43 (t, 4H), 1.24-1.31 (s, 8H).

^{1}H-NMR of HDA (500 MHz, DMSO) δ (ppm): 6.17-6.22 (q, 2H), 6.03-6.07 (q, 2H), 5.53-5.56 (q, 2H), 3.08-3.12 (m, 4H), 1.39-1.43 (t, 4H), 1.24-1.31 (m, 4H).

Synthesis of oligo(amidoamine)s OAAs:

Synthesis of OAAs: The OAAs was synthesized via the Michael-addition polymerization of secondary amine groups TMDP with the vinyl groups of alkylene diacrylamide (Scheme S1). To obtain the OAA structures as Scheme S1, the molar ratio

of diamine moiety and vinyl moieties was 40:1. The synthesis process of T-D compound was as follows: TMDP (30.0 g) was dissolved in MeOH (100 mL), and then DDA (1.0 g) were added to this solution. Reaction was performed at 50°C for 1 day. The product was obtained by pouring the reactant mixture into an excess of acetone. The purification of the reaction products was performed 3 times with a reprecipitation method using chloroform as a solvent and acetone as a non-solvent. The resulting precipitate was dried under vacuum at 40°C over 48 h to give a final yield of 1.7 g.

^{13}C NMR (500 MHz, $CDCl_3$) of OAA: Fig. S1.

Maldi-tof ($CHCl_3$) of OAA: Fig. S2. T-D molecular weight (Mw) of 701.4 Da is relative to molecular weight of two TMDP and one DDA confirming the formation of T-D structure in Scheme S1. It is similar for T-O, T-H and T-M and their molecular weights are 673.6, 645.5 and 575.4 Da, respectively.

Characterization:

^{13}C and ^{1}H NMR (Varian Unity Inova 500NB, 500 Mhz) was used to examine the structure of OAAs in $CDCl_3$ and DDA, ODA, and HDA in DMSO, respectively. Structure of OAAs was further confirmed by Maldi-tof (Voyager Elite MALDI-TOF MS, Perkin-Elmer PerSteptive Biosystems, Framingham, MA) using 337 nm N_2 laser. Fourier transform infrared spectroscopy (FTIR, Nicolet 380, ATR technique) was used to determine the hydrogen bond of amide moieties in D_2O (10 and 20 wt%). Morphology of T-O xerogel was analyzed by a scanning electron microscope (SEM, Hitachi S-2140). Xerogel was prepared by free-drying the T-O hydrogel at pH 7.4 under vacuum in liquid nitrogen for 12 h.[2]

Sol-gel phase diagram:

The sol (flow)-gel (no flow) phase transition was recorded using the test inverting method.[1,3] 4 mL vial test tubes containing of OAA solutions (0.5 mL) was immersed in a temperature-controllable water bath. Each sample at a given concentration (e.g., 10 wt%)

was dissolved in water. The pH of the solution was then adjusted to the required pH (e.g., 7.0, 7.2 and 7.4) with 5M HCl or 5M NaOH aqueous solutions at room temperature. The sol-gel transition was determined by inverting the vial. The sample was considered to be a gel if there was no flow observed within 1 min. At the temperature intervals of 2 °C, the samples were equilibrated for 20 min.

G' versus time of T-O gelator at concentration of 20 wt% (pH 7.4 and 37ºC) is illustrated in Figure S 3. As shown in Figure S3, the system reached equilibrium state within 10 min. Thus at temperature intervals of 2ºC, the equilibration of the samples in 20 min are reasonable.

Rheology measurements:

The variation of the viscosity of the OAA compounds water (20 wt%) as a function of pH and temperature was determined with dynamic mechanical analysis (Bohlin Rotational Rheometer). The OAA solution was placed between two 20 mm diameter plates with a gap of 0.5 mm. The controlled stress and frequency were 0.4 Pa and 1 rad s^{-1} respectively. The heating rate was 0.2 °C min^{-1}. A stress-amplitude sweep experiment was performed at a constant oscillation frequency of 1 Hz at 37°C. To determine thermoreversibility of OAA gelators, dynamic mechanical analysis of T-O gel at pH 7.4 was carried out. Heating and cooling rate were 0.2 °C min^{-1} (Figure S4). Frequency dependence of moduli was carried out at a stress of 0.4 Pa and 37°C (Figure S6).

Gel morphology in vivo:

A male Sprague-Dawley (SD) rat (Hanlim Experimental Animal Laboratory, Seoul, Korea) was used to observe the gelation of the OAA compounds in vivo. The SD rat (5–6 weeks old, average body weight 200 g) was cared for in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication 85-23, revised 1985).

To perform the injectability and in vivo spontaneous gel formation, a 200 μL T-O solution (20 wt%) at pH 6.6 and 20°C was subcutaneously injected into a SD rat with a syringe needle. After 5 min of injection, the SD rat was sacrificed to determine the morphology. Gel formation in the rat due to an increase in pH and temperature of the tissue fluid surrounding the injection site suggests the gelator solution can be easily injected into the body and forms an in situ gel within a short time (Figure S4).

References

1 M. K. Nguyen, D. K. Park and D. S. Lee, *Biomacromolecules,* 2009, **10**, 728.

2 N. Shi, H. Dong, G. Yin, Z. Xu and S. Li, *Adv. Funct. Mater*, 2007, **17**, 1837.

3 K. Dayananda, C. He and D. S. Lee, *Polymer,* 2008, **49,** 4620.

Scheme S1. Schematic diagram of the synthesis and chemical structure of the OAAs.

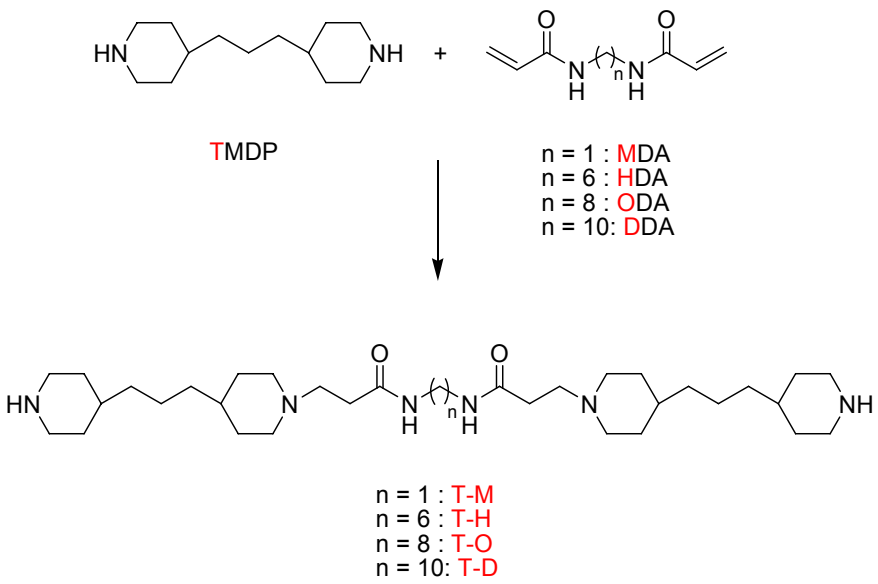


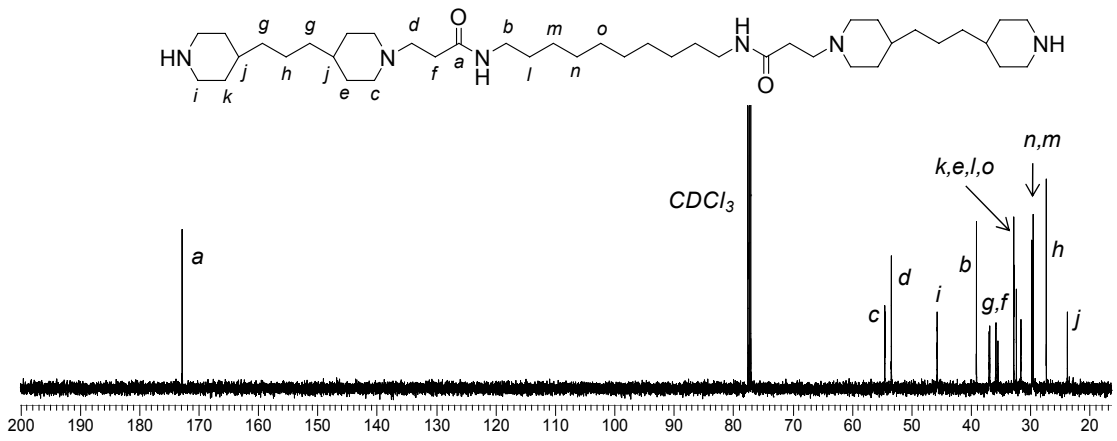
Table S1. Gelation concentration of OAA gelators at pH 7.4.

OAA gelator	Gelation concentration (wt%)
T-M	10[a]
T-H	10
T-O	5
T-D	5

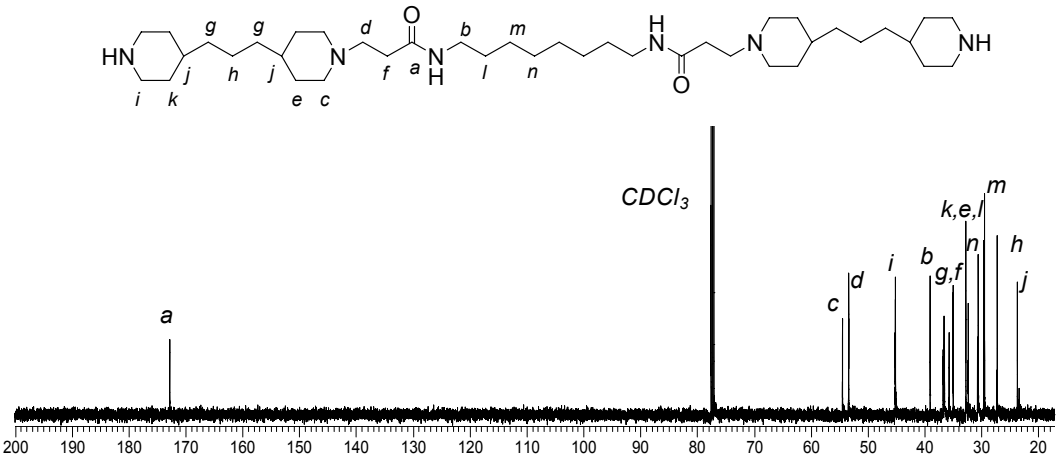
[a] gelation only occurred at pH 7.7.

Figure S1. ^{13}C NMR spectra a) T-D, b) T-O, c) T-H, d) T-M.

a)



b)



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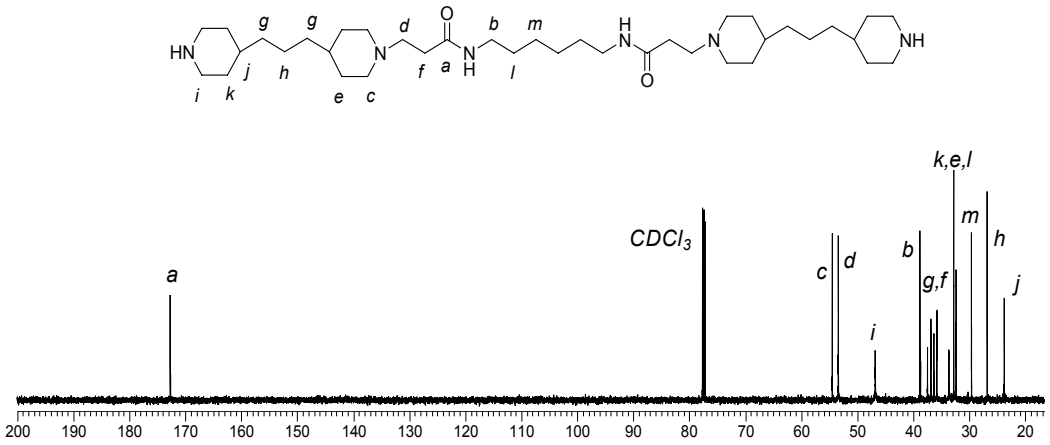
HN
NH
N
O
H
i k j h g g e c d f a b l m
CDCl3
a
c d i b g,f m k,e,l h j
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d)

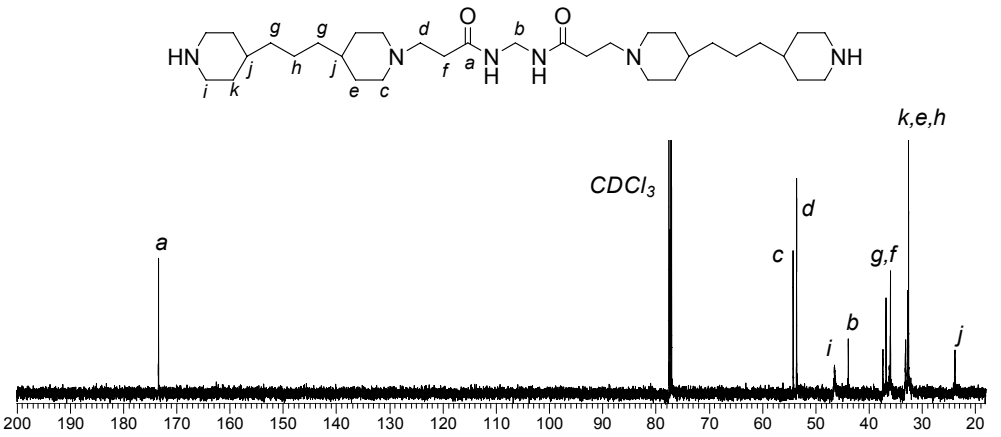

HN
NH
N
O
H
i k j h g g e c d f a b
CDCl3
a
c d i b g,f k,e,h j
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

Figure S2. Maldi-tof spectrum of T-D.

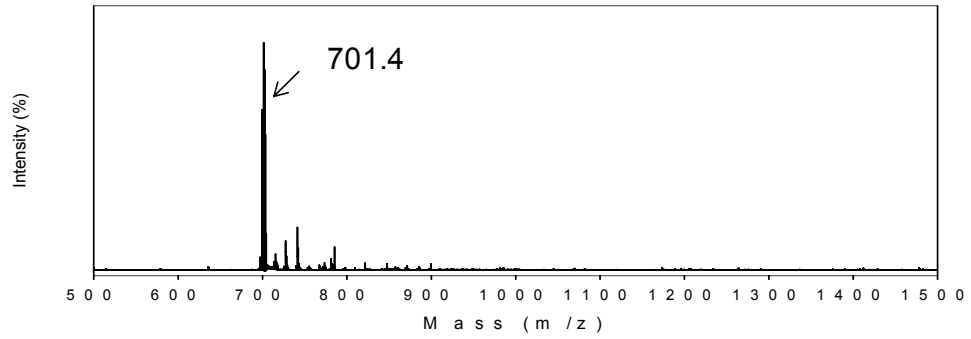


Figure S3. G' versus time of T-O gelator at concentration of 20 wt% (pH 7.4 and 37ºC)

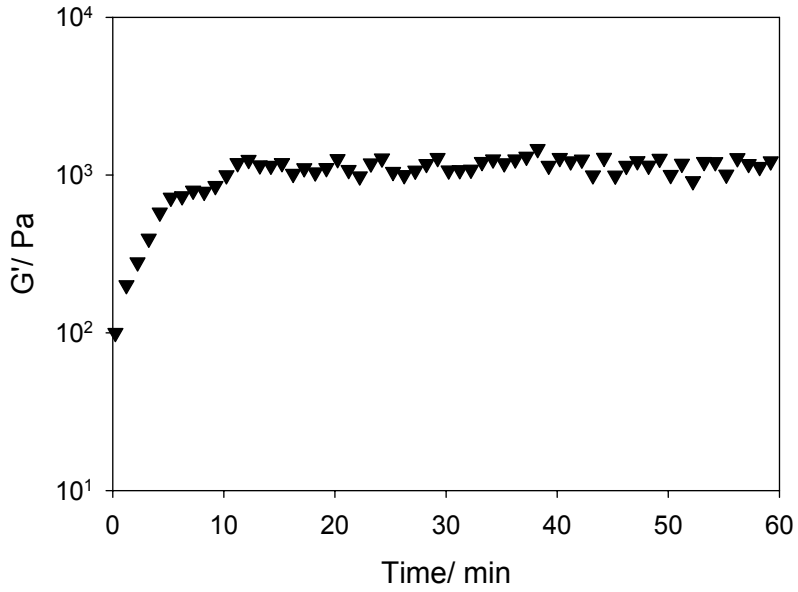


Figure S4. Thermoreversibility of T-O hydrogel (10 wt%) at pH 7.4 with two heating-cooling circles.

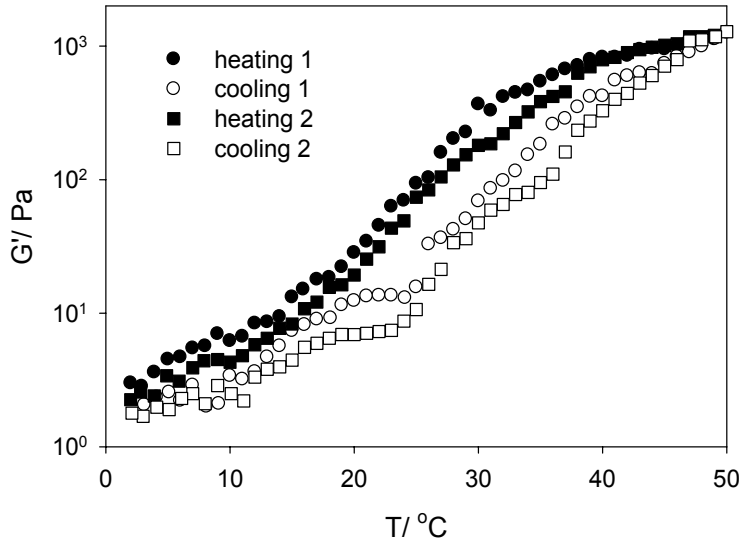
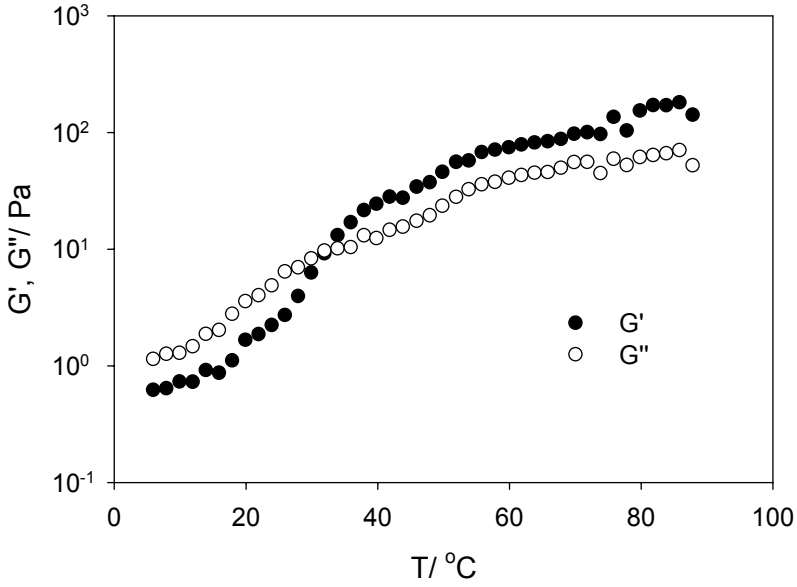


Figure S5. G' and G" changes of T-O solution (20 wt%) as a function of temperature at different pH values a) pH 7.0, b) pH 7.4.

a)



b)

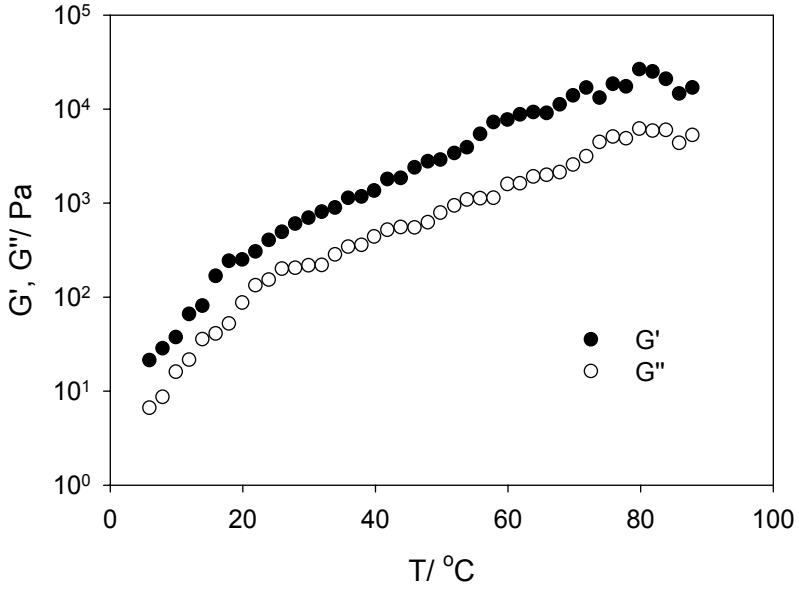


Figure S6. G' versus frequency of the hydrogels (10 wt%, pH 7.4) at different OAA structures.

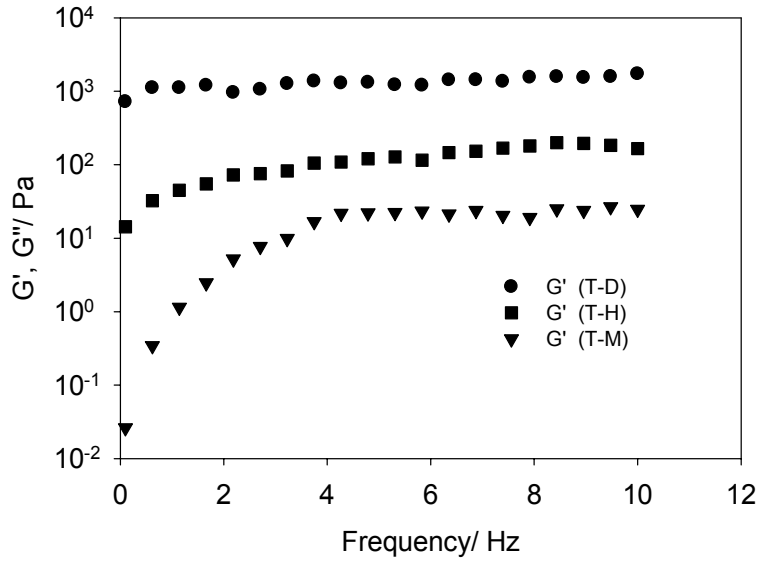


Figure S7. Gel morphology after 5 min injection of T-O solution (20 wt%) into a rat.