Title: "Non-swellable" hydrogel without mechanical hysteresis

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Abstract: Hydrogels are three-dimensional polymer networks that contain a large amount of water inside. Certain hydrogels can be injected in solution and transformed into the gel state with the required shape. Despite their potential biomedical applications, the use of hydrogels has been severely limited, because all the conventional hydrogels inevitably "swell" under physiological conditions, which drastically degrades their mechanical properties. We report the synthesis of injectable "non-swellable" hydrogels from hydrophilic and thermoresnponsive polymers, in which two independently occurring effects (i.e., swelling and shrinking) oppose each other. The hydrogels can endure a compressive stress up to 60 MPa and can be stretched more than seven-fold without hysteresis. Our results demonstrate that the suppression of swelling helps retain the mechanical properties of hydrogels under physiological conditions.

Main Text: Hydrogels are used as scaffolds for tissue engineering (*1*), temporary supports for cells (*2*), and vehicles for drug delivery system (*3*). Although specially engineered hydrogels are known to exhibit excellent physical propertie (*4-7*), they may have limited applicability, because conventional hydrogels "swell" as a result of the difference in osmotic pressure. Swelling drastically weakens the mechanical toughness. Further, in the case of hydrogels that exhibit hysteresis while being deformed, the equilibrium between osmotic and elastic energies is inevitably lost, when the polymer network is partly (or even temporarily) broken. This occurrence consequently leads to swelling (*8*) and a continual mechanical load eventually destroys the hydrogels. This phenomenon commonly occurs in engineered gels made of natural components (*9-11*), self-healing gels (*12-16*), which are based on non-covalent cross-links, and exceptionally tough double network gels (*4, 8*), which utilize sacrificial bonds.

Our strategy to achieve a robust hydrogel that can operate under physiological conditions is based on the control of swelling by means of the introduction of thermoresponsive segments, which collapse above a certain critical temperature (T_c) due to predomination of hydrophobic interactions (*17*), into the polymer network. We designed and fabricated hydrogels composed of tetra-armed hydrophilic and thermoresponsive polymer units (Fig. 1). The cross-linking reaction is based on the mutually reactive functional end-groups (i.e., active ester and amino end-groups). Here, the molar amount of active ester end-groups always equals to that of amino end-groups. We can prepare hydrogels with the required shape simply by mixing aqueous solutions of the polymer units and the resultant hydrogels contain a high amount of water (89.8-90.4%) in the as-prepared state. No organic solvent, catalyst, or ultraviolet radiation is involved in the preparation step. The gelation time

after injection can be controlled from seconds to hours (Fig. S1), which allows for a range of potential applications. The swelling behavior can be controlled through a selection of the proper ratio of polymer units without losing the ideal network structure (Fig. 1D). The homogeneous network structure contributes to the high deformability (*7*). Furthermore, because the polymer network is made of covalent cross-links and has no special energy dissipation mechanism, the hydrogels do not undergo hysteresis during the deformation process.

The swelling behavior is regulated by the thermoresponsive segment ratio (r); for example, when $r = 0$, the hydrogel is composed only of hydrophilic segments (Fig. 1D, left). An alternating structure is formed when $r = 0.5$ (Fig. 1D, right). Figure 2A shows the swelling ratio (Q) of hydrogels with different r values. All the hydrogels, which were prepared at 10ºC, swelled in an aqueous environment at 10ºC ($Q \approx 300\%$). However, the hydrogels drastically changed their volume around their T_c (approximately 25ºC, irrespective of r). This control over swelling at a fixed T_c cannot be achieved with conventional thermoresponsive hydrogels that are fabricated by a random copolymerization of hydrophilic and thermoresponsive monomer; T_c increases significantly with an increase in the amount of hydrophilic monomer and can exceed 37ºC (*18*). Based on the volume change, the water content also decreased; the degree of water release depended on r (Fig. 2C). In the same manner as conventional hydrogels, the swelling ratio for $r = 0$ is greater than 100% over the whole temperature range, indicating that the hydrogel swells and alters its original shape in an aqueous environment. Although the hydrogel with $r = 0.4$ also swelled at 10ºC, the hydrogel recovered its original shape at approximately 37ºC ($Q \approx 100\%$) (Fig. 2A). Here, it is important to note that the hydrogels with $r = 0.4$ still retain a high amount of water at 37ºC ($W \approx 90\%$) (Fig. 2C). Further, unlike other conventional hydrogels with similar compositions that often induce turbidity (*19-21*), our hydrogels retain their transparency even above T_c, irrespective of r (Fig. 2B), which suggests that the hydrogels have a homogeneous network structure.

Due to the unique polymer network structure, the hydrogels exhibit an anomalous water allotment between hydrophilic and thermoresponsive segments. We estimated the swelling ratios of the thermoresponsive (Q_t) and swollen phases (Q_h), assuming that the water content of the thermoresponsive segments at 40ºC (W_t) is an intrinsic value for each chemical compound ($W_t \approx 12\%$) (*supplementary online text*). Although the hydrophilic segments are highly hydrated even at 40ºC ($Q_h > 135\%$), the swelling ratio decreased with an increase in r (Fig. 2D). When the two segments independently hold water molecules, Q_h should be constant irrespective of r. This r-dependent swelling ratio suggests that the water molecules in the hydrophilic segments are also expelled by the shrinkage of the thermoresponsive segments.

The mechanical properties of hydrogels are strongly affected by their degree of swelling. To examine the general effect of swelling, we performed elongation tests with different r values after the hydrogels reached their equilibrium-swollen state in D-PBS at 37ºC. The representative stress-elongation curves showed that the maximum elongation ratio (λ_{max}) diminished with a decrease in r (Fig. 3A). This decrease in λ_{max} can be explained by the following definition: λ_{max} of polymer gels is defined as the ratio of two lengths of network strands — the fully stretched and initial states (*22*). Because "swelling" prestretches the network strands of the initial state, λ_{max} inevitably decreases.

The hydrogel with $r = 0.4$, in which the swelling is suppressed, showed improved mechanical properties. Although a conventional hydrogel ($r = 0$) in the equilibrium-swollen state was easily torn off before the hydrogel was stretched three-fold, the hydrogel with $r = 0.4$ did not rupture even after being stretched more than seven-fold (Fig. 3B). In addition, the "non-swellable" hydrogel showed practically no hysteresis, at least when stretched less than

four-fold, which indicates that the elongation did not break the covalent bonds of the polymer network (Fig. 3C). This reversible feature is prominent when compared with the hydrogels that exhibit hysteresis during deformation, which essentially fail to tolerate a continual mechanical load. In contrast to such hydrogels, our hydrogels showed no swelling or weakening in aqueous media even after a repetitive mechanical overload. Notably, the "non-swellable" hydrogel endured a compressive stress of up to 60 MPa even though the hydrogel was in its equilibrium-swollen state (Fig. 3D), which is comparable to so-called "tough hydrogels" previously reported (*4-7*), whereas the swollen hydrogel (r = 0) fractured at a strain of ~80%, showing a maximum stress of ~0.4 MPa.

The properties of the hydrogels can thus be tuned through the selection of r, which will give specific values for extensibility, breakage strength or elasticity. The hydrogels can be further adjusted via the introduction of other functional polymer units. For example, the hydrogels can be used as biodegradable materials for certain applications by introducing a cleavable polymer unit (Fig. S2). The degradation profiles of the modified hydrogels are simply regulated by the amount of cleavable polymer units (Fig. S3); hydrogels with a higher amount of cleavable linkages are subject to faster degradation. Our results demonstrate that the swelling suppression of hydrogels may help maintain their initial shape and retain their mechanical properties under physiological conditions.

References and Notes:

1. K. Y. Lee, D. J. Mooney, Hydrogels for tissue engineering. *Chem Rev* **101**, 1869 (Jul, 2001).
2. G. D. Nicodemus, S. J. Bryant, Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B-Re* **14**, 149 (Jun, 2008).
3. A. S. Hoffman, Hydrogels for biomedical applications. *Adv Drug Deliver Rev* **54**, 3 (Jan 17, 2002).
4. J. P. Gong, Y. Katsuyama, T. Kurokawa, Y. Osada, Double-network hydrogels with extremely high mechanical strength. *Adv Mater* **15**, 1155 (2003).
5. K. Haraguchi, T. Takehisa, Nanocomposite hydrogels: A unique organic-inorganic network structure with extraordinary mechanical, optical, and swelling/de-swelling properties. *Adv Mater* **14**, 1120 (2002).
6. Y. Okumura, K. Ito, The polyrotaxane gel: A topological gel by figure-of-eight cross-links. *Adv Mater* **13**, 485 (2001).
7. T. Sakai *et al.*, Design and fabrication of a high-strength hydrogel with ideally homogeneous network structure from tetrahedron-like macromonomers. *Macromolecules* **41**, 5379 (2008).
8. T. Nakajima, T. Kurokawa, S. Ahmed, W. L. Wu, J. P. Gong, Characterization of internal fracture process of double network hydrogels under uniaxial elongation. *Soft Matter* **9**, 1955 (2013).
9. P. A. Janmey *et al.*, The Mechanical-Properties of Actin Gels - Elastic-Modulus and Filament Motions. *J Biol Chem* **269**, 32503 (1994).
10. S. M. Mithieux, J. E. J. Rasko, A. S. Weiss, Synthetic elastin hydrogels derived from massive elastic assemblies of self-organized human protein monomers. *Biomaterials* **25**, 4921 (2004).
11. C. Storm, J. J. Pastore, F. C. MacKintosh, T. C. Lubensky, P. A. Janmey, Nonlinear elasticity in biological gels. *Nature* **435**, 191 (2005).
12. E. A. Appel, J. del Barrio, X. J. Loh, O. A. Scherman, Supramolecular polymeric hydrogels. *Chem Soc Rev* **41**, 6195 (2012).

13. K. Haraguchi, K. Uyama, H. Tanimoto, Self-healing in Nanocomposite Hydrogels. *Macromol Rapid Comm* **32**, 1253 (2011).
14. A. Phadke *et al.*, Rapid self-healing hydrogels. *P Natl Acad Sci USA* **109**, 4383 (2012).
15. J. Y. Sun *et al.*, Highly stretchable and tough hydrogels. *Nature* **489**, 133 (2012).
16. Q. Wang *et al.*, High-water-content mouldable hydrogels by mixing clay and a dendritic molecular binder. *Nature* **463**, 339 (2010).
17. M. Heskins, J. E. Guillet, Solution Properties of Poly(N-isopropylacrylamide). *Journal of Macromolecular Science: Part A - Chemistry* **2**, 1441 (1968/12/01, 1968).
18. J. E. Chung, M. Yokoyama, T. Aoyagi, Y. Sakurai, T. Okano, Effect of molecular architecture of hydrophobically modified poly(N-isopropylacrylamide) on the formation of thermoresponsive core-shell micellar drug carriers. *J Control Release* **53**, 119 (1998).
19. J. Cui, M. A. Lackey, G. N. Tew, A. J. Crosby, Mechanical Properties of End-Linked PEG/PDMS Hydrogels. *Macromolecules* **45**, 6104 (2012).
20. J. Li *et al.*, Self-assembled supramolecular hydrogels formed by biodegradable PEO-PHB-PEO triblock copolymers and alpha-cyclodextrin for controlled drug delivery. *Biomaterials* **27**, 4132 (2006).
21. S. Reinicke *et al.*, Smart hydrogels based on double responsive triblock terpolymers. *Soft Matter* **5**, 2648 (2009).
22. S. P. Obukhov, M. Rubinstein, R. H. Colby, Network Modulus and Superelasticity. *Macromolecules* **27**, 3191 (1994).

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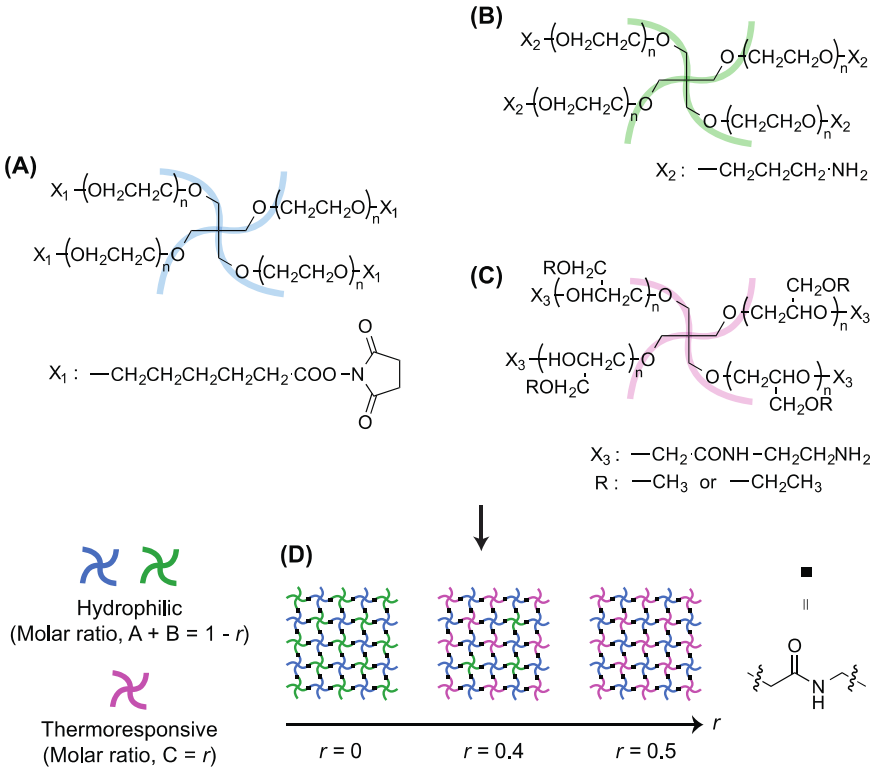

Fig. 1.

Schematic of the hydrogel system. (A) Tetra-armed poly(ethylene glycol) with active ester end-groups. **(B)** Tetra-armed poly(ethylene glycol) with amino end-groups. **(C)** Tetra-armed poly(ethyl glycidyl ether-*co*-methyl glycidyl ether) with amino end-groups. **(D)** Polymer network composed of hydrophilic (blue) and thermoresponsive (pink) polymer units where *r* represents the thermoresponsive segment ratio. Junction amide bonds (■).

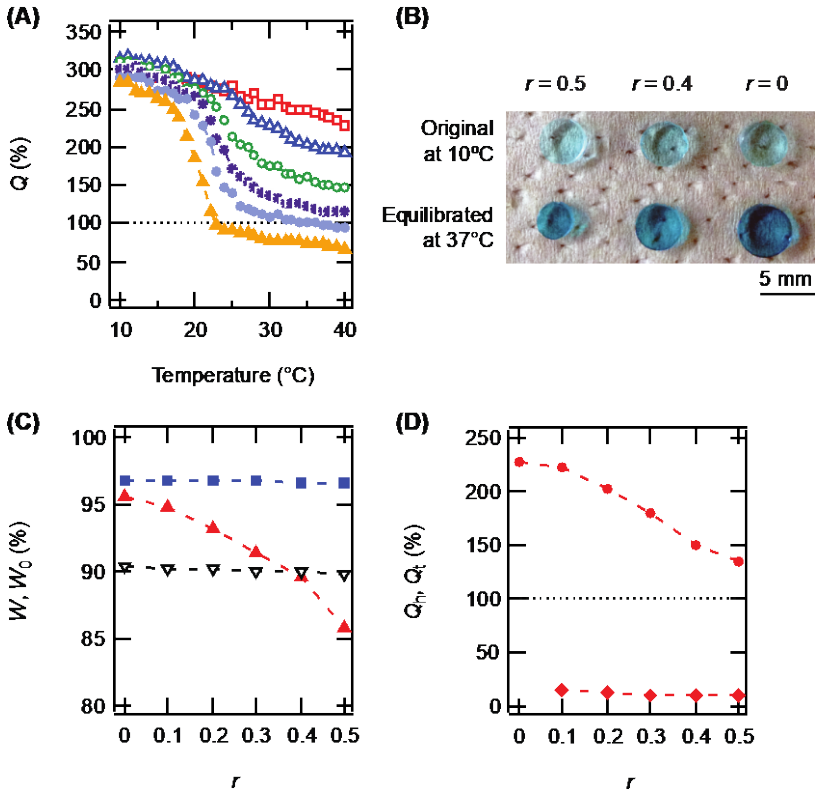

Fig. 2.
Swelling behavior of the hydrogels in Dulbecco's phosphate-buffered saline (D-PBS). **(A)** The swelling ratio (Q) as a function of temperature. The symbols represent the thermoresponsive segment ratio (r); r = 0 (□), 0.1 (△), 0.2 (○), 0.3 (*), 0.4 (●), and 0.5 (▲). $Q = V/V_0 \times 100$, where V is the volume of the samples in the equilibrium-swollen state at each temperature and V_0 is the initial volume of the samples. The dotted line is a guide to the eye for 100%. **(B)** Photos of hydrogels that exhibit different swelling degrees depending on r; as-prepared samples at 10ºC (top) and samples equilibrated in D-PBS at 37ºC (bottom). The transparent hydrogels were colored only for visibility. **(C)** Equilibrium water content (W) of hydrogels at 10ºC (■) and 40ºC (▲). Initial water content (W_0) of the as-prepared samples (▽). $W = (V - V_p) / V \times 100$, where V_p is the total volume of polymers. **(D)** The swelling ratios in the hydrophilic (Q_h, ●) and thermoresponsieve (Q_t, ◆) segments at 40ºC. $Q_h = V_h/V_{h\_0} \times 100$, where V_h is the volume of the hydrophilic segment in the equilibrium-swollen state at 40ºC and V_{h_0} is the initial volume of the hydrophilic segment. $Q_t = V_t/V_{t\_0} \times 100$, where V_t is the volume of the thermoresponsive segment in the equilibrium-swollen state at 40ºC and V_{t_0} is the initial volume of the thermoresponsive segment. The dotted line is a guide to the eye for 100%.

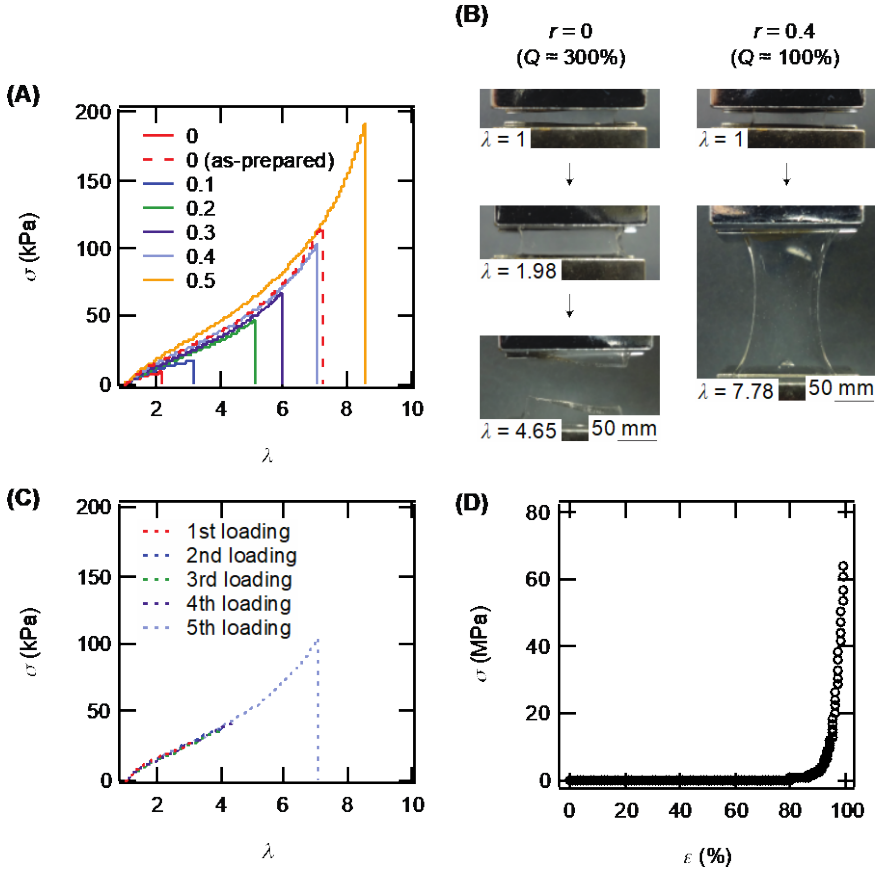

Fig. 3.
Mechanical properties of hydrogels equilibrated in D-PBS at 37°C. (A) Representative stress–elongation curves. The colors represent the thermoresponsive segment ratio (r). Only for r = 0 (as-prepared), the samples were measured immediately after taken from the mold. **(B)** Photos of hydrogels (r = 0 and 0.4) during elongation tests. **(C)** Stress–elongation curves of hydrogel (r = 0.4). **(D)** The results of a compression test (r = 0.4). ε represents strain.

Supplementary Materials:

Materials and Methods

Schemes S1-S4

Figures S1-S3