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**Osmosis-Induced Mechanical Disintegration of Polymer Network
Gels**

(浸透圧駆動型力学エネルギーによる高分子網目ゲルの崩壊)

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Chapter 1: General Introduction

1.1 Overview

A polymer network is a three-dimensional structure formed by polymers that are crosslinked via chemical (covalent bond) or physical (ionic interactions or entanglements) crosslinks. The physical properties of polymer networks vary depending on factors such as the chemical species of the constituent monomers and the crosslinking density.[1, 2] And these factors enable them to be shaped into a wide range of materials. Polymer networks are widely encountered in everyday life, for instance, in tires, soft contact lenses, and ion exchange resins. It is no exaggeration to say that many aspects of modern life rely on the utility of these polymer network materials.

One key unique feature that sets polymer networks apart from traditional materials like metals or ceramics is their open system—that is, their ability to exchange matter with the external environment.[3–5] When a polymer network is immersed into a good solvent, the polymer network absorbs solvent molecules and expands in volume, and this process are well-known as swelling. This swelling is driven by an osmotic pressure difference that arises from the concentration difference between inside and outside the polymer network. However, the polymer network cannot diffuse out into the external solvent because they are crosslinked. As a result, the network swells instead—this is the fundamental mechanism behind swelling.[6]

When a polymer network contains a large amount of absorbed solvent, it is referred to as a polymer gel (hereafter, simply "gel"). Gels have bio-tissue like physical properties like high water content, deformability, and low friction.[7, 8] And they are expected to be biocompatible materials. Accordingly, numerous studies have investigated the application of gels as biomaterials, for instance, as fillers for tissue defects or as actuators.[9–13] It is quite

important to understand the relationship between the macroscopic mechanical behavior of the swollen gel and the microscopic stretched state of its network strands to ensure that implanted gels exhibit the desired mechanical properties and functionalities within the body. Ideally, one should be able to predict and control gel properties over a wide range of swelling states. To achieve this, it is essential to understand swelling behavior of gels.

Then arises the fundamental question: To what extent can a gel swell in a solvent, and what determines this swelling limit? From the structural perspective of polymer networks, the potential swelling range of a gel may be considered span from the fully contracted state to the fully stretched state of the network strands. However, in reality, gels does not swell until the network chains reach their maximal stretched state. Rather, swelling does not proceed exceed at a certain regime—this is known as the equilibrium swelling state. A gel has the equilibrium swelling state depending on its network structure, solvent type, temperature, and external constraints.[14] According to the theory proposed by Flory and Rehner, the total swelling pressure of a gel, π_{total} , can be divided into the elastic pressure, π_{el} , which works to suppress swelling, and the osmotic pressure, π_{os} , which works to promote swelling.[15–17] The elastic pressure originates from the elastic energy stored in the gel network.[17] The osmotic pressure consists of a mixing contribution, π_{mix} , which is based on the free energy of mixing of the gel network and solvent, and an ionic contribution, π_{ion} , which originates from the difference of the mobile counterion concentration between the inside and outside of the gel.[15, 16] When the elastic and osmotic pressures are balanced, gels reaches its swelling equilibrium and the following equation holds:

$$\pi_{\text{total}} = -\pi_{\text{el}} + (\pi_{\text{mix}} + \pi_{\text{ion}}) = 0. \quad (\text{Equation 1})$$

At this equilibrium state, the free energy of the system is minimized, and further swelling beyond this equilibrium becomes thermodynamically unfavorable (**Figure 1-1**). As a result, gels rarely reach an excessively swelling state beyond its equilibrium in typical conditions. Also reproducing such excessively swollen gels experimentally has long been a challenge. Consequently, most previous studies on the relationship between swelling and mechanical properties of gels have been restricted to the equilibrium or lower swelling regime, and the mechanical behavior of excessively swollen gels remains largely unexplored.[18–21]

To overcome this thermodynamic limitation and access the excessively swelling regime, the molecular stent method has been invented. [22–24] In this method, dense linear polymers—referred to as molecular stents—are introduced into the interest gel (**Figure 1-2**). A gel with introduced molecular stents is termed a St gel. Unless these molecular stents form chemical crosslinks or strong physical interactions with the original gel network, their contribution to the gel's elasticity is negligible. However, the introduced molecular stents significantly increase the osmotic pressure, π_{os} , of the system. This method enables independent tuning of osmotic pressure without affecting the elastic pressure from the original gel network. As a result, the increased osmotic pressure allows the St gel to absorb additional solvent molecules, and St gels reach a new swelling equilibrium state beyond that of the native gel network alone. This method successfully enables the experimental realization of excessively swollen gels where the states were previously inaccessible.

Although the molecular stent method has succeeded in expanding the experimentally accessible swelling range, the previous method only introduced molecular stents once. Consequently, molecular stents concentration in the St gels was limited and insufficient to induce excessively swelling up to the structural swelling limit where the most

network strands are maximally stretched. In this study, we aim to swell the gel network excessively around the structural swelling limit or even beyond it, by employing a multi-step molecular stent method. In this approach, molecular stents are introduced multiple times, thereby incrementally increasing the stent concentration within the gel (**Figure 1-3**). We successfully demonstrated, for the first time, the systematic swelling of gels across the entire structurally allowable swelling range. Furthermore, we report a previously unobserved phenomenon, which we term "*osmotic explosion*"—a catastrophic collapse of the gel caused by network strand scission when the gel swells excessively beyond its structural swelling limit.

1.2 Outline of this dissertation

Chapter 2 first derives the expressions for osmotic pressure and elastic pressure and summarizes their relationships at the equilibrium swelling state. Subsequently, we provide an overview of the background of research on the correlation between swelling degree and gel properties, and describe a method for swelling gels around the structural swelling limit. One such method to swell gel network beyond the equilibrium swelling is the molecular stent method. A gel with molecular stents is called an St gel. The introduced molecular stents significantly increase the osmotic pressure of the St gel. This enables independent control of the gel's osmotic pressure apart from the elasticity of the original gel network. Due to the elevated osmotic pressure induced by the molecular stents, the St gel absorbs more solvent molecules in a good solvent, and reaches a new swelling equilibrium beyond that of the original gel network. This method has successfully expanded the experimentally accessible swelling range of gels. However, conventional molecular stent method have been limited in the achievable concentration of introduced molecular stents into gels, which has proven insufficient for reaching the structural swelling limit. To address this issue, this study aims to achieve excessive swelling of the gel network around its structural swelling limit or even beyond via multi-step molecular stent method. Enough high dense molecular stents of different chemical species are introduced repeatedly in multiple steps.

Chapter 3 reports the mechanical property changes with swelling toward the structural swelling limit and the bulk collapse observed beyond this limit. In this study, molecular stents were introduced into the gel in three successive steps. The gel introduced with molecular stents twice (referred to as St² gel) exhibited a swelling ratio of approximately 7–8 times (uniaxial swelling ratio) in water, which is very close to the structural swelling limit

estimated based on the monomer length and bond angle of the network strands. The St^2 gel, swollen nearly to the structural swelling limit, showed a stiffness approximately 2–3 times greater than that of the as-prepared original gel state. Furthermore, the elastic energy stored per network strand in the swollen gel was estimated from the gel's elastic modulus. The results revealed that the elastic energy per strand in the St^2 gel was almost three orders of magnitude greater than that in the as-prepared original gel state, suggesting that the stiffening observed near the structural swelling limit reflects rigid behavior of the highly stretched network strands. Next, the gel into which molecular stents were introduced three times (St^3 gel) was immersed in water and swelled them beyond the structural swelling limit. Approximately three hours after immersion, the St^3 gels exhibited swelling from the surface and ruptured into microgels—a phenomenon we call “*osmotic explosion*.” Numerous microgels ranging from sub-millimeter to several millimeters in size were formed following osmotic explosion, and their surfaces were covered with many fine and coarse cracks. This osmotic explosion is considered to result from rupture of overstretched network strands driven by excessive osmotic pressure generated by the highly concentrated molecular stents.

Chapter 4 discusses the conditions under which osmotic explosion—i.e., gel collapse beyond the structural swelling limit—occurs, as well as the mechanism of microgel formation. The condition for osmotic explosion was evaluated based on the elastic energy stored in the network strands. Let U denote the elastic energy stored per strand estimated from the elastic modulus of the gels, and $U_{\max}$ the maximum energy that single strand can withstand estimated from the bond dissociation energy.[25] Osmotic explosion was found to occur when the following inequality holds:

$$U \geq U_{\max}. \quad (\text{Equation 2})$$

This relationship was found to hold regardless of the preparation conditions of the original gel before molecular stent introduction. Potential causes of the hierarchical disintegration behavior observed in the St^3 gel include leakage of molecular stents during the disintegration process, stress concentration due to the molecular weight distribution of the network strands, and differential swelling rates between the gel surface and interior.

Chapter 5 summarizes the findings of this study and discusses future perspectives.

This work is the first to systematically realize the full structurally allowable swelling range of gels and to report osmotic explosion beyond the structural swelling limit. Furthermore, it demonstrates that osmotic pressure can exert sufficient force to destruct and process polymer network materials, suggesting potential applications in addressing environmental issues associated with polymer disposal. The findings are also expected to contribute to the advancement of osmotic-pressure-driven mechanochemistry by sophisticating this method since the force applied to network strands can be controlled via the swelling degree.

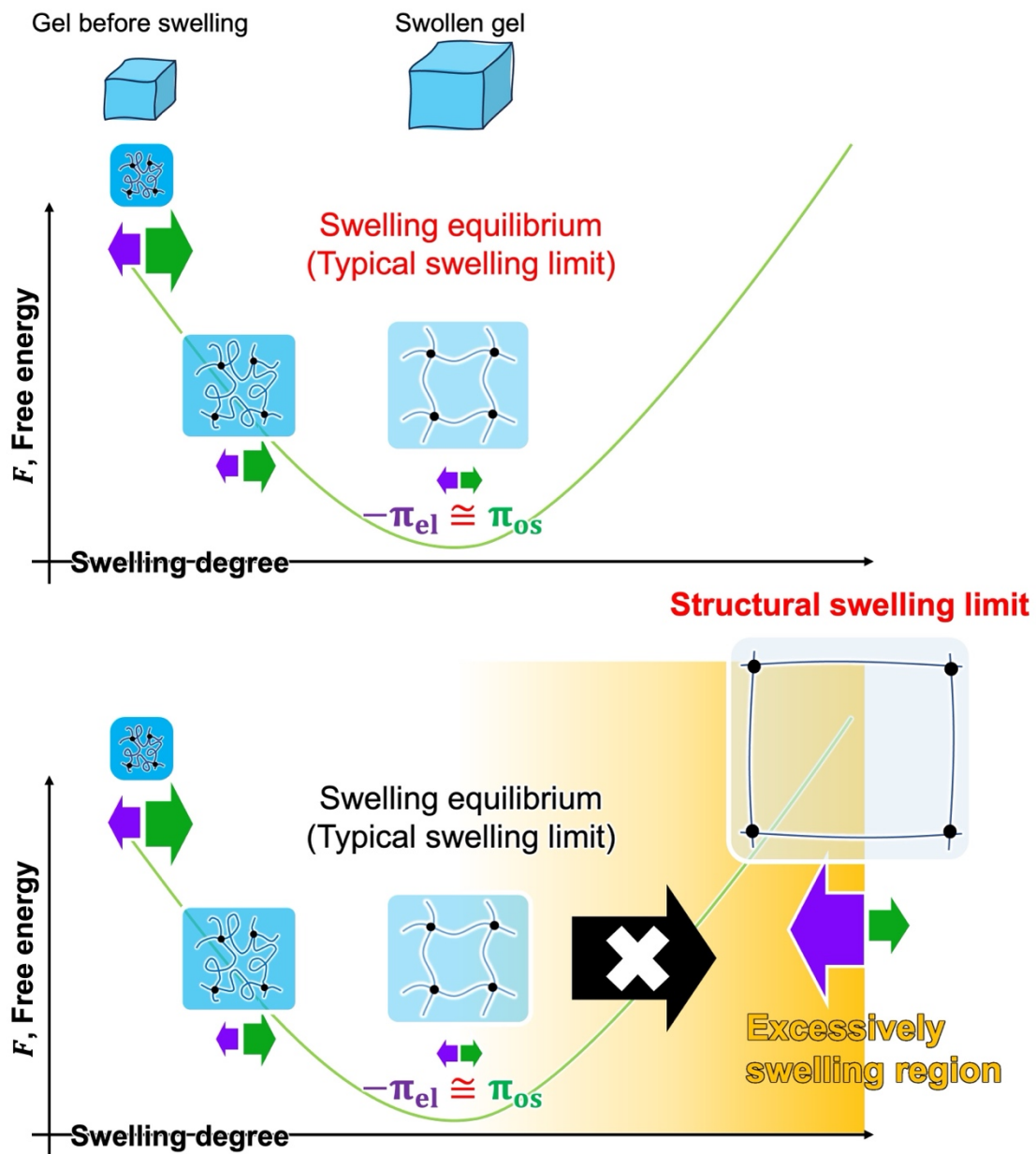


Figure 1-1. The relationship between osmotic pressure, π_{os} , and elastic pressure, π_{el} with swelling process. π_{os} promote swelling, while π_{el} suppress swelling. When the π_{os} and π_{el} are balanced, gels reaches equilibrium swelling and swelling stops. Typically, gels cannot reach the structural swelling limit where the network strands are fully stretched.

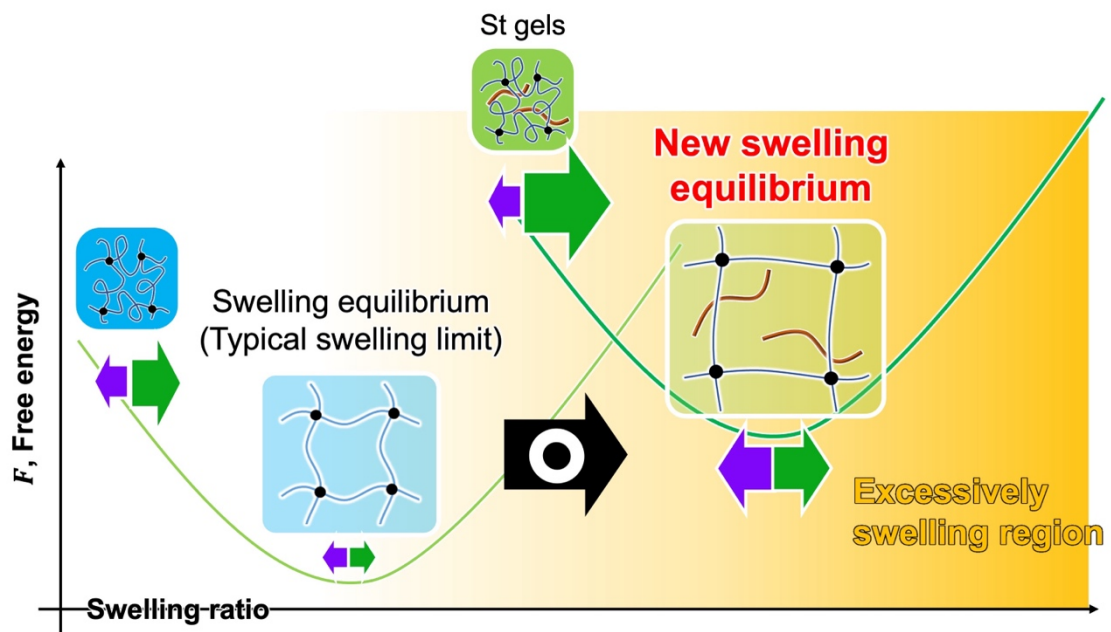


Figure 1-2. Schematic illustration of conventional molecular stent method. Osmotic pressure can be tuned independent of elasticity of the original gel network by this method, because molecular stent does not form strong interaction with the original gel network. St gels (gels with molecular stent) excessively swell and reach new equilibrium swelling state in a good solvent.

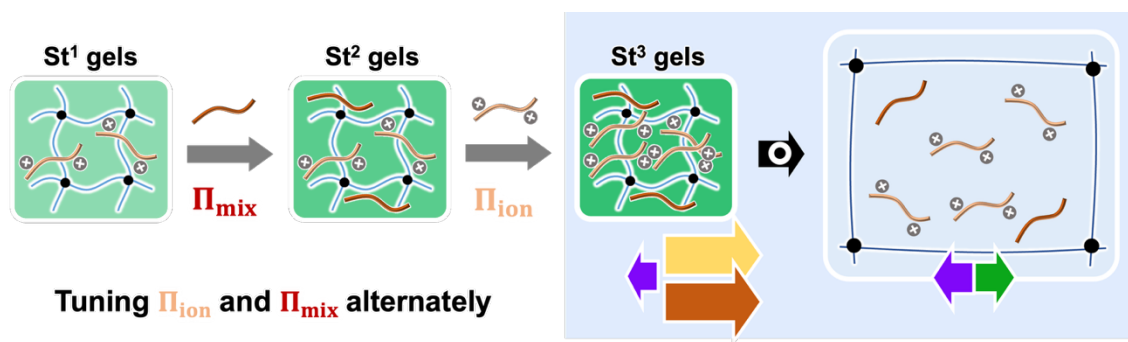


Figure 1-3. Schematic illustration of multi-step molecular stent method. Different chemical species of linear polymer are introduced alternately in the target gels. This method can increase the concentration of molecular stent and swelling ratio of the St gels more effectively than the conventional one.

1.2 References

1. Kwang Suk Oh, Jeong Seok Oh, Hyun Seok Choi and Young Chan Bae “Effect of Cross-Linking Density on Swelling Behavior of NIPA Gel Particles” *Macromolecules*, **31**, 7328–7335 (1998).
2. Rachel Shet Hui Wong, Mark Ashton and Kalliopi Dodou “Effect of Crosslinking Agent Concentration on the Properties of Unmedicated Hydrogels” *Pharmaceutics*, **7**, 305–319 (2015).
3. Oliver Lieleg and Katharina Ribbeck “Biological Hydrogels as Selective Diffusion Barriers” *Trends Cell Biol.*, **21**, 543–551 (2011).
4. Brandon V. Slaughter, Shahana S. Khurshid, Omar Z. Fisher, Ali Khademhosseini and Nicholas A. Peppas “Hydrogels in Regenerative Medicine” *Adv. Mater.* **21**, 3307–3329 (2009).
5. Jianyu Li and D. J. Mooney “Designing hydrogels for controlled drug delivery” *Nat Rev Mat.* **1**:16071 (2016).
6. Takamasa Sakai “Physics of Polymer Gels” Ed.; Wiley-VCH: Weinheim, 2020.
7. Jian Ping Gong, Takayuki Kurokawa, Tetsuharu Narita, Go Kagata, Yoshihito Osada, Goro Nishimura and Masataka Kinjo “Synthesis of Hydrogels with Extremely Low Surface Friction” *J. Am. Chem. Soc.* **123**, 5582–5583 (2001).
8. Jian Ping Gong “Friction and lubrication of hydrogels—its richness and complexity” *Soft Matter* **2**, 544–552 (2006).
9. Qiang Shi, Hao Liu, Deding Tang, Yuhui Li, XiuJun Li and Feng Xu “Bioactuators based on stimulus-responsive hydrogels and their emerging biomedical applications” *NPG Asia Materials* **11**:64 (2019).

10. Mingtong Li, Xin Wang, Bin Dong and Metin Sitti “In-air fast response and high speed jumping and rolling of a light-driven hydrogel actuator” *Nature Communications* **11**:3988 (2020).
11. Shohei Ishikawa, Yasuhide Iwanaga, Takashi Uneyama, Xiang Li, Hironori Hojo, Ikuo Fujinaga, Takuya Katashima, Taku Saito, Yasushi Okada, Ung-il Chung, Naoyuki Sakumichi and Takamasa Sakai “Percolation-induced gel–gel phase separation in a dilute polymer network” *Nature Materials* **22**, 1564–1570 (2023).
12. Sidi A. Bencherif, R. Warren Sandsa, Deen Bhattaa, Praveen Arany, Catia S. Verbekea, David A. Edwards and David J. Mooney “Injectable preformed scaffolds with shape-memory properties” *PNAS* **109**, 19590–19595 (2012).
13. Neda Latifi, Meisam Asgari, Hojatollah Vali and Luc Mongeau “A tissue-mimetic nano-fibrillar hybrid injectable hydrogel for potential soft tissue engineering applications” *Scientific reports* **8**:1047 (2018).
14. Quesada-Pérez M., Maroto-Centeno J. A., Forcadab J. and Hidalgo-Alvarez R. “Gel swelling theories: the classical formalism and recent approaches” *Soft Matter* **7**, 10536–10574 (2011).
15. Flory P. J. “*Principles of Polymer Chemistry*” (Cornell Univ. Press, Ithaca, 1953, pp. 458–470, 577–579).
16. Rubinstein M. and Colby R. H. “*Polymer Physics*” (Oxford Univ. Press, Oxford, 2003, pp.140–146).
17. Flory P. J. and Rehner J. “Statistical Mechanics of Cross-Linked Polymer Networks I. Rubberlike Elasticity” *J. Chem. Phys.* **11**, 521–520 (1943).
18. Obukhov S. P., Rubinstein M. and Colby R. H. “Network Modulus and Superelasticity” *Macromolecules* **27**, 3191–3198 (1994).

19. Horkay F., Tasaki I. and Bassar P. J. “Osmotic Swelling of Polyacrylate Hydrogels in Physiological Salt Solution” *Biomacromolecules* **1**, 84–90 (2000).
20. Sakai T., Kurakazu M., Akagi Y., Shibayama M. and Chung U.-I. “Effect of swelling and deswelling on the elasticity of polymer networks in the dilute to semi-dilute region” *Soft Matter* **8**, 2730–2736 (2012).
21. Tanaka T. “Collapse of Gels and the Critical Endpoint” *Phys. Rev. Lett.* **40**, 820–823 (1978).
22. Nakajima T., Sato H., Zhao Y., Kawahara S., Kurokawa T., Sugahara K. and Gong J. P. “A Universal Molecular Stent Method to Toughen any Hydrogels Based on Double Network Concept” *Adv. Funct. Mater.* **22**, 4426–4432 (2012).
23. Matsuda T., Nakajima T., Fukuda Y., Hong W., Sakai T., Kurokawa T., Chung U.-I. and Gong J. P. “Yielding Criteria of Double Network Hydrogels” *Macromolecules* **49**, 1865–1872 (2016).
24. Hoshino K., Nakajima T., Matsuda T., Sakai T. and Gong J. P. “Network elasticity of a model hydrogel as a function of swelling ratio: from shrinking to extreme swelling state” *Soft matter* **14**, 9693–9701 (2018).
25. Lake G. J. and Thomas A. G. “The Strength of Highly Elastic Materials” *Proc. Roy. Soc. A* **300**, 108–119 (1967).

Chapter 2: Background

2.1 Theoretical framework of osmotic and elastic pressure

As described in Section 1.1, when a gel is immersed into a suitable solvent, it absorbs the solvent and swells up to reduce the osmotic pressure difference between the inside and outside of the gel. The gel reaches an equilibrium swelling when the osmotic pressure, π_{os} , balances with the elastic pressure, π_{el} . [1-3] In the following sections, we describe in detail the derivation of π_{mix} and π_{ion} , and establish the corresponding relationship among these components at equilibrium swelling.

We first describe the method for calculating π_{mix} . The mixing contribution due to polymer–solvent mixing, π_{mix} , can be determined by the Flory–Huggins lattice model.

Assuming that the volume change upon mixing between the polymer network and solvent is negligible, the change in Helmholtz free energy due to mixing, ΔF_{mix} , can be expressed as:

$$\frac{\Delta F_{mix}}{nkT} = \frac{\varphi}{N} \ln \varphi + \varphi_s \ln \varphi_s + \chi \varphi \varphi_s. \quad (\text{Equation 3})$$

Here, n is the total number of lattice sites in the system, and n is related to the total volume, denoted as V and the volume per lattice site, denoted as v_s , by the following relation

$V = n \cdot v_s$. Now, the lattice volume v_s is defined as the volume of a water molecule,

$v_s \approx 3 \times 10^{-29} \text{ m}^3$. N is the number of lattice sites occupied by a single polymer and

corresponds to the polymerization degree. φ and φ_s are the volume fractions of the polymer and solvent, respectively, and satisfy the following relation $\varphi_s = 1 - \varphi$. χ is the Flory

interaction parameter between the polymer and solvent. Assuming that polymerization degree is enough large (i.e., $N \rightarrow \infty$), Equation 3 simplifies to:

$$\frac{\Delta F_{mix}}{nkT} \cong \varphi_s \ln \varphi_s + \chi \varphi \varphi_s. \quad (\text{Equation 4})$$

π_{mix} is obtained by differentiating the mixing free energy change ΔF_{mix} with respect to volume V . Since the volume change of the system is assumed to occur only due to the migration of solvent (water) molecules, we write:

$$dV = v_s dn_s. \quad (\text{Equation 5})$$

where n_s is the number of lattice sites occupied by water molecules. Accordingly, π_{mix} is given by:

$$\pi_{\text{mix}} = -\frac{1}{v_s} \frac{\partial F_{\text{mix}}}{\partial n_s} \cong -\frac{kT}{v_s} \{\ln \phi_s + \phi + \chi \phi (1 - \phi_s)\}. \quad (\text{Equation 6})$$

Equation 6 is applicable only to binary systems, such as those in which a gel composed of a single type of polymer chain swells by absorbing water. In this study, however, two types of molecular stents with different chemical species are introduced into the gel, making it a quaternary system. Therefore, the following discussion extends the formulation to a four-component system.[4] Based on Equation 3, the change in Helmholtz free energy due to the mixing of three types of polymers and one solvent is expressed as:

$$\begin{aligned} \frac{\Delta F_{\text{mix}}}{nkT} = & \frac{\phi_1}{N_1} \ln \phi_1 + \frac{\phi_2}{N_2} \ln \phi_2 + \frac{\phi_3}{N_3} \ln \phi_3 + \phi_s \ln \phi_s + \chi_{12} \phi_1 \phi_2 + \chi_{13} \phi_1 \phi_3 + \chi_{23} \phi_2 \phi_3 + \chi_{1s} \phi_1 \phi_s \\ & + \chi_{2s} \phi_2 \phi_s + \chi_{3s} \phi_3 \phi_s. \end{aligned} \quad (\text{Equation 7})$$

Here, N_i is the number of lattice sites occupied by a single molecule of i , ϕ_i is the volume fraction of component i , and χ_{ij} is the Flory interaction parameter between components i and j , where $i, j=1, 2, 3$, and s represent the original polymer network, 1st molecular stent, 2nd molecular stent, and water molecules, respectively. 1st molecular stent and 3rd molecular Assuming that the polymerization degree of molecular stents are also enough large, as in the case of the polymer network, Equation 7 simplifies to:

$$\begin{aligned} \frac{\Delta F_{mix}}{nkT} \cong & \varphi_s \ln \varphi_s + \chi_{12} \varphi_1 \varphi_2 + \chi_{13} \varphi_1 \varphi_3 + \chi_{23} \varphi_2 \varphi_3 + \chi_{1s} \varphi_1 \varphi_s + \chi_{2s} \varphi_2 \varphi_s \\ & + \chi_{3s} \varphi_3 \varphi_s. \end{aligned} \quad (\text{Equation 8})$$

Following the same derivation procedure as in Equation 6, the osmotic pressure contribution from mixing, π_{mix} , is given by:

$$\begin{aligned} \pi_{mix} = & -\frac{1}{v_s} \frac{\partial F_{mix}}{\partial n_s} \\ \cong & -\frac{kT}{v_s} \{ \ln \varphi_s + \varphi_1 + \varphi_2 + \varphi_3 - \chi_{12} \varphi_1 \varphi_2 - \chi_{13} \varphi_1 \varphi_3 - \chi_{23} \varphi_2 \varphi_3 + \chi_{1s} \varphi_1 (1 - \varphi_s) \\ & + \chi_{2s} \varphi_2 (1 - \varphi_s) + \chi_{3s} \varphi_3 (1 - \varphi_s) \}. \end{aligned} \quad (\text{Equation 9})$$

Next, we describe the method for calculating ionic contribution, π_{ion} , which originates from the ionic molecular stents introduced into the gel. For the estimation of π_{ion} , we assume that only the concentration difference of mobile counterions contributes to π_{ion} and the effect of repulsion of the fixed ions are negligible. Under this assumption, π_{ion} of the St gels immersed in water, originating from the introduced ionic polymer as the molecular stent, can be calculated by using the following equation; [4]

$$\pi_{ion} \cong kT \frac{\alpha \varphi_1}{v_1}. \quad (\text{Equation 10})$$

Here, α is the ratio of mobile counterions to the total ionic polymer concentration, and v_1 is the volume of the monomer unit of ionic molecular stent. The value of α can be estimated from the Donnan potential measurement of the St gel immersed into the dilute salt aqueous solution with the following equation; [5, 6]

$$\alpha = \frac{C_s}{\frac{zF\Phi}{10^{2.3RT}} C_g}. \quad (\text{Equation 11})$$

Here, z , F , R , and T are the valence of the mobile ion in consideration, the Faraday constant, the gas constant, and the absolute temperature, where the value are $z = 1$, $F = 9.65 \times 10^4$ C/mol, $R = 8.31$ J/mol · K, and $T = 298$ K, respectively. C_s is the monovalent salt concentration

of outside solution of the gels, Φ is the Donnan potential between the outside solution and the gels, and C_g is the total counterion concentration inside the St gels.

As described above, Equation 1 holds at the equilibrium swelling state. Previous studies have reported that the elastic pressure π_{el} can be estimated from the elastic modulus E of the gel as:

$$\pi_{el} \cong -G = -E/3. \quad (\text{Equation 12})$$

Therefore, at equilibrium swelling, the following expression is valid:

$$\begin{aligned} \pi_{os} = \pi_{mix} + \pi_{ion} &= -\frac{E}{3} \\ &\cong -\frac{kT}{v_s} \{ \ln \varphi_s + \varphi_1 + \varphi_2 + \varphi_3 - \chi_{12} \varphi_1 \varphi_2 - \chi_{13} \varphi_1 \varphi_3 - \chi_{23} \varphi_2 \varphi_3 + \chi_{1s} \varphi_1 (1 - \varphi_s) \\ &\quad + \chi_{2s} \varphi_2 (1 - \varphi_s) + \chi_{3s} \varphi_3 (1 - \varphi_s) \} + kT \frac{\alpha \varphi_1}{v_1}. \end{aligned} \quad (\text{Equation 13})$$

2.2 Physical properties change with swelling

The mechanical properties of as-prepared gel differ from those of the same gel after swelling in a solvent. This is because swelling reduces the number density of network strands while simultaneously stretching them. For a swollen gel—such as one implanted in the body—to exhibit its intended functionality, it is desirable to be able to predict the mechanical property changes with swelling. Although many studies have investigated changes in elastic modulus and the rate of stimuli responsiveness with swelling ratio, these discussions have primarily been based on changes in the number density of network strands.[7–10] In contrast, little attention has been given to the stretching degree of network strands. The main reason for this is thought to lie in the inherent structural heterogeneity of the polymer network structure within conventional gels.

2.2-1 Tetra-PEG gels: A platform for investigating the structure–property relationship

Gels synthesized via free-radical polymerization typically exhibit a broad distribution in the polymerization degree between crosslinking points of the network strands. As a result, when such gels swell, both highly stretched and loosely coiled strands coexist within the network, leading to heterogeneous swelling at the microscopic level. In gels with such structural inhomogeneity, it is difficult to correlate the macroscopic swelling ratio with the microscopic stretching ratio of network strands. This network inhomogeneity makes it extremely difficult to establish a clear relationship between the stretching ratio of strands and the mechanical properties of the gel.

However, in 2008, Sakai *et al.* reported the development of Tetra-PEG gels, which possess a relatively uniform network structure.[11, 12] Tetra-PEG gels can be easily synthesized by mixing two types of tetra-functional prepolymers with reactive end groups. By

tuning the molecular weight and concentration of the prepolymers when preparation, polymerization degree between crosslinks and the extent of structural defects of the network can be precisely controlled. Since this pioneering work, numerous studies have employed Tetra-PEG gels, highlighting the interest in understanding the relationship between internal network structures and gel properties.[13–16] As mentioned above, Tetra-PEG gels exhibit a remarkably narrow distribution in the polymerization degree between crosslinks. Consequently, the network strands are expected to swell uniformly unlike conventional gels. Therefore it becomes possible to quantitatively correlate the mechanical properties of the gel, swelling ratio, and the network strands stretching ratio by utilizing Tetra-PEG gel.

2.2-2 Molecular stent method to exceed the equilibrium swelling

Numerous studies, including those on Tetra-PEG gels, have discussed the correlation between swelling degree and gel properties change. However, the majority of these studies have been limited to the equilibrium swelling state or lower swelling regime. As described in previous chapter, the elastic pressure, π_{el} , balances with the osmotic pressure, π_{os} , at swelling equilibrium. When a gel swells beyond this equilibrium, a high elastic pressure is generated due to the accumulated elastic energy in the highly stretched network strands. However, conventional gels typically do not possess sufficient osmotic pressure to overcome this elevated elastic pressure. As a result, excessively swelling of gels is almost impossible without special manipulation. Consequently, the mechanical properties of such an excessively swollen gels have not been extensively studied.

To achieve excessively swelling, it is necessary to increase the osmotic pressure independently of the elasticity originating from the original gel network. For this purpose, the molecular stent method was developed by Nakajima *et al.*[17] In this method, linear polymers—either ionic or nonionic—are introduced into the gels. These polymers are

termed molecular stents, in analogy to medical stents used to expand blood vessels. Unless molecular stents do not form chemical crosslinks with the original gel network and interact physically, they behave as dangling chains within the gel and are considered to have negligible impact on its elasticity. When such a stent-introduced gel, St gel, is immersed in a good solvent, the osmotic pressure generated by the molecular stents allows the St gels to absorb additional solvent and swelling excessively. The St gels reaches a new equilibrium state where the osmotic pressure balances with the elastic pressure from the stretched network strands.

An additional advantage of the molecular stent method is that the type of osmotic pressure component— π_{mix} or π_{ion} —can be selectively tuned. π_{mix} is proportional to the concentration of nonionic polymers (or to the blob concentration in the semi-dilute regime), while π_{ion} is proportional to the concentration of mobile counterions (see Section 2.1 for details). Typically, π_{ion} can generate osmotic pressures approximately 100 times greater than π_{mix} .^[4] Although gels composed of nonionic polymers generally exhibit limited swelling depending on their preparation conditions, introducing ionic polymers as molecular stents enables efficient excessively swelling even in such gels. In this way, the molecular stent method is applicable regardless of the chemical species of the gels. Indeed, there are reports in which the combination of Tetra-PEG gels with the molecular stent method has enabled the quantitative coupling of swelling degree, stretching ratio of network strands, and gel elasticity.^[18]

2.3 Multi-step molecular stent method to achieve swelling around the structural swelling limit

If we assume that the microscopic stretching limit of the network strands corresponds to the macroscopic swelling limit of the gel, then the gel should be able to swell from the most contracted state of the strands (i.e., the dried state) to their fully stretched limit. Although the molecular stent method has made it possible to access this previously inaccessible excessively swelling regime, research on excessively swollen gels remains limited. Also no study has yet succeeded in experimentally realizing a gel in which the network strands are stretched to their maximum limit. This is because the conventional molecular stent method has limitation on the concentration of stents that can be introduced into the gel, thereby making it difficult to generate sufficiently high osmotic pressure to stretch the network strands to their limit.[17, 18]

In this study, we aim to induce excessively swelling such that the network strands approach their stretching limit and to investigate the mechanical properties of the gel in that state. To achieve this, we adopt a multi-step molecular stent method to generate higher osmotic pressure. Specifically, we alternately introduce ionic and nonionic polymers as molecular stents, thereby promoting efficient stepwise increases in osmotic pressure. As described in Section 2.2, π_{ion} can generate significantly higher osmotic pressure than π_{mix} . One might assume that repeated increases in π_{ion} alone would suffice to induce excessively swelling to the structural swelling limit. However, it is important to note that π_{ion} arises from the concentration difference of mobile counterions between the inside and outside of the gel. When a gel with ionic molecular stents is immersed again in a precursor solution for ionic molecular stents, the concentration gradient of mobile counterions become small. This results in a significant reduction of π_{ion} . Consequently, the gel does not swell so much and cannot efficiently absorb

additional precursor monomers for ionic molecular stents, thereby limiting the final concentration of ionic molecular stents within the gel (**Figure 2-1**). In contrast, when a St gel with nonionic molecular stents is immersed in a precursor solution for ionic molecular stents, it can swell effectively for π_{mix} . As a result, the gel can absorb a larger amount of precursor monomers for ionic molecular stents, ultimately allowing for a higher internal concentration of ionic molecular stents.

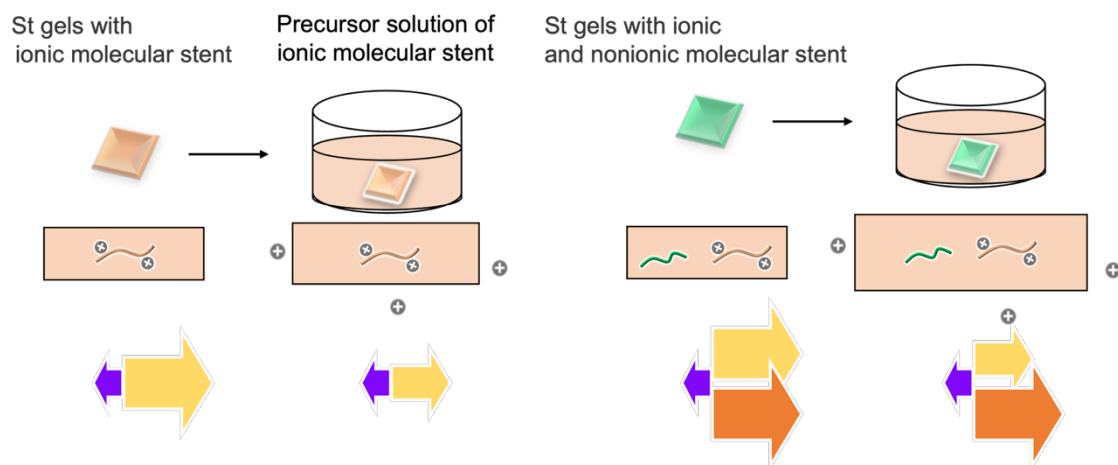


Figure 2-1. Illustration showing how multi-step molecular stent method enables dense molecular stent introduction. St gels with ionic molecular stent cannot swell so much in the ionic molecular stent precursor solution due to reduction of π_{ion} . Thus, the St gels cannot absorb enough amount of precursor of molecular stent. However, St gels with ionic and nonionic molecular stent can swell sufficiently in the ionic molecular stent precursor solution, due to π_{mix} even if π_{ion} becomes small. Thus, the St gels can absorb enough amount of precursor of molecular stent.

2.4 References

1. Flory P. J. “*Principles of Polymer Chemistry*” (Cornell Univ. Press, Ithaca, 1953, pp. 458–470, 577–579).
2. Rubinstein M. and Colby R. H. “*Polymer Physics*” (Oxford Univ. Press, Oxford, 2003, pp.140–146).
3. Flory P. J. and Rehner J. “Statical Mechanics of Cross-Linked Polymer Networks I. Rubberlike Elasticity” *J. Chem. Phys.* **11**, 521–520 (1943).
4. Nakajima T., Chida T., Mito K., Kurokawa T. and Gong J. P. “Double-network gels as polyelectrolyte gels with salt-insensitive swelling properties” *Soft Matter* **16**, 5487–5496 (2020).
5. Guo H., Kurokawa T., Takahata M., Hong W., Katsuyama Y., Luo F., Ahmed J., Nakajima T., Nonoyama T. and Gong J. P. “Quantitative Observation of Electric Potential Distribution of Brittle Polyelectrolyte Hydrogels Using Microelectrode Technique” *Macromolecules* **49**, 3100–3108 (2016).
6. Nishimura T., Guo H., Kiyama R., Katsuyama Y., Gong J. P. and Kurokawa T. “In Situ Evaluation of the Polymer Concentration Distribution of Microphase Separated Polyelectrolyte Hydrogels by the Microelectrode Technique” *Macromolecules* **54**, 10776–10785 (2021).
7. Obukhov S. P., Rubinstein M. and Colby R. H. “Network Modulus and Superelasticity” *Macromolecules* **27**, 3191–3198 (1994).
8. Urayama K. and Kohjiya S. “Crossover the concentration dependence of swelling and elastic properties of polysiloxane networks crosslinked in solution” *J. Chem. Phys.* **104**, 3352–3359 (1996).

9. Kovac J. “Modified Gaussian Model for Rubber Elasticity” *Macromolecules* **11**, 362–365 (1978).
10. Hong W., Zhao X., Zhou J. and Suo Z. “A theory of coupled diffusion and large deformation in polymeric gels” *J. Mech. Phys. Solids* **56**, 1779–1793 (2008).
11. Sakai T., Matsunaga T., Yamamoto Y., Ito C., Yoshida R., Suzuki S., Sasaki N., Shibayama M. and Chung U.-I. “Design and Fabrication of a High-Stretch Hydrogel with Ideally Homogeneous Network Structure from Tetrahedron-like Macromonomers” *Macromolecules* **41**, 5379–5384 (2008).
12. Sakai T. “Gelation mechanism and mechanical properties of Tetra-PEG gel” *React. Funct. Polym.* **73**, 898–903 (2013).
13. Matsunaga T., Sakai T., Akagi Y., Chung U.-I. and Shibayama M. “Structure Characterization of Tetra-PEG gel by Small-Angle Neutron Scattering” *Macromolecules* **42**, 1344–1351 (2009).
14. Akagi Y., Katashima T., Katsumoto Y., Fujii K., Matsunaga T., Chung U.-I. and Shibayama M., Sakai T. “Examination of the Theories of Rubber Elasticity Using an Ideal Polymer Network” *Macromolecules* **44**, 5817–5821 (2011).
15. Lange F., Schwenke K., Kurakazu M., Akagi Y., Chung U.-I., Lang M., Sommer J. U., Sakai T. and Saalwächter K. “Connectivity and Structural Defects in Model Hydrogels: A Combined Proton NMR and Monte Carlo Simulation Study” *Macromolecules* **44**, 9666–9674 (2011).
16. Akagi Y., Gong J. P., Chung U.-I. and Sakai T. “Transition between Phantom and Affine Network Model Observed in Polymer Gels with Controlled Network Structure” *Macromolecules* **46**, 1035–1040 (2013).
17. Nakajima T., Sato H., Zhao Y., Kawahara S., Kurokawa T., Sugahara K. and Gong J. P. “A Universal Molecular Stent Method to Toughen any Hydrogels Based on Double Network

Concept” *Adv. Funct. Mater.* **22**, 4426–4432 (2012).

18. Hoshino K., Nakajima T., Matsuda T., Sakai T. and Gong J. P. “Network elasticity of a model hydrogel as a function of swelling ratio: from shrinking to extreme swelling state” *Soft matter* **14**, 9693–9701 (2018).

Chapter 3: Strategy to Achieve Excessively Swelling around the Structural Swelling Limit

3.1 Introduction

As polymer gels of interest, we used Tetra-PEG gels with well-regulated network structures to correlate the macroscopic and microscale deformations (**Figure 3-1**). [1, 2] As described in the previous section, Tetra-PEG gels are synthesized via end-linking reactions of four-armed polyethylene glycols with narrow molecular weight distributions, resulting in network strands with controlled lengths and low spatial inhomogeneity. Due to this well controlled structure, it is reasonable to assume that the macroscopic deformation of Tetra-PEG gels with swelling is well coupled with the molecular-scale deformation of the network strands. [3–5] In other words, if the gel swells by an uniaxial swelling ratio of λ_s , the network strands are also expected to be stretched approximately by λ_s . In this study, Tetra-PEG gels were prepared using prepolymers with various molecular weights and concentrations. The preparation conditions of the Tetra-PEG gels are denoted by the average molecular weight of the prepolymers, M_w , and the prepolymer concentration in the pre-gel solution relative to their overlap concentration, C^* .

To achieve excessively swelling far beyond the original swelling equilibrium, we employed a multi-step molecular stent method, in which ionic and nonionic molecular stents were introduced alternately (**Figure 3-2**). Although various types of polymers can be used as molecular stents, we used poly(2-acrylamido-2-methylpropane sulfonic acid sodium salt) (PNaAMPS) and polyacrylamide (PAAm) as the ionic and nonionic molecular stents, respectively. These polymers commonly used in hydrogel synthesis. Into the Tetra-PEG gels of interest, we first introduced the ionic PNaAMPS as the 1st molecular stent, followed by the

nonionic PAAm as the 2nd molecular stent, and again the ionic PNaAMPS as the 3rd molecular stent. Hereafter, the Tetra-PEG gels with the first, second, and third molecular stents are referred to as St¹ (PNaAMPS), St² (PNaAMPS/PAAm), and St³ (PNaAMPS/PAAm/PNaAMPS) gels, respectively. This alternate introduction of ionic and nonionic molecular stents generally increases the swelling ratio of Tetra-PEG gels more effectively than repeatedly introducing only the ionic stent (**Figure 3-4**).

3.2 Experimental-Materials and Methods-

3.2-1 Fabrication of the Tetra-PEG gels

The Tetra-PEG gels were synthesized via cross-end coupling reaction of their prepolymers. Tetra amine-terminated PEG (PA-PEG, NOF Corporation) was dissolved in phosphate buffer (pH 7.4), while tetra NHS-terminated PEG (HS-PEG, NOF Corporation) was dissolved in citrate phosphate buffer (pH 5.8). The average molecular weight, M_w of each prepolymers was either 10,000 g mol⁻¹ (10 k) or 20,000 g mol⁻¹ (20 k). For the 10 k system, the prepolymer concentrations relative to their overlap concentration ($C^* = 75$ g L⁻¹) were set to 0.86 C^* , C^* , 1.26 C^* , or 1.4 C^* . For the 20 k system, the relative concentrations (with $C^* = 40$ g L⁻¹) were 0.75 C^* , C^* (40 g L⁻¹), 1.5 C^* , or 2 C^* . The two prepolymer solutions were mixed and injected into a glass mold using a plastic syringe equipped with a filter membrane. The rectangular glass mold was constructed using two glass plates and a silicone rubber spacer (0.5 or 3.0 mm thick) with an inner area of 80 × 80 mm². The molds filled with the mixed prepolymer solution were sealed and left at room temperature for 24 hours to complete the reaction. Some of the resulting Tetra-PEG gels were wrapped and stored at 5 °C, while the rest were immersed in water for 24 hours to prepare equilibrium swollen Tetra-PEG gels.

3.2-2 Fabrication of the St-PEG gels

The St-PEG gels were prepared by the introduction of ionic or nonionic molecular stents. The precursor solution for the ionic stents (used for the 1st and 3rd molecular stents) was prepared by dissolving 2 M 2-acrylamido-2-methylpropanesulfonic acid sodium salt (NaAMPS; Toa Gosei Co., Ltd.) and 0.1 mol% (to the monomer) 2-oxoglutaric acid (α -keto; Wako Pure Chemical Industries, Ltd.), which serves as a radical initiator, in water. The precursor solution for the nonionic stent (used for the 2nd molecular stent) was prepared by dissolving 3 M

acrylamide (AAM; Wako Pure Chemical Industries, Ltd.) and 0.5 mol% (to the monomer) α -keto in water.

To fabricate St¹-PEG gels, equilibrium swollen Tetra-PEG gels were immersed in the ionic precursor solution for 24 hours, picked up the gels from the solution, then sandwiched them between a pair of glass plates. The sandwiched gels were irradiated with UV light (peak wavelength: 365 nm) for 6 hours in a glove box filled with Ar gas to synthesize of linear PNaAMPS (1st molecular stent) within the Tetra-PEG gels.

To fabricate St²-PEG gels, the as-prepared St¹-PEG gels were immersed in the nonionic precursor solution for 24 hours, and PAAM (2nd molecular stent) was synthesized inside the St¹-PEG gels using the same UV irradiation procedure.

To fabricate St³-PEG gels, the as-prepared St²-PEG gels were again immersed in the ionic precursor solution for 24 hours, and PNaAMPS (3rd molecular stent) was synthesized within the St²-PEG gels using the same procedure.

The resulting St¹, St², and St³-PEG gels were stored at 5 °C in sealed plastic bags to prevent drying.

3.2-3 Measurement of uniaxial swelling ratio of the gels

As-prepared Tetra-PEG gels, St¹-PEG gels, and St²-PEG gels were immersed in water for 24 hours to reach their swollen states, which were regarded as the equilibrium swelling states. The thickness of the gels was measured using a thickness gauge (SDA-25, KOBUNSHI KEIKI Co., Ltd.). The uniaxial swelling ratio of a swollen gel, λ_s , was defined as:

$$\lambda_s = \frac{t}{t_0}. \quad (\text{Equation 14})$$

Here t is the thickness of the swollen $\text{St}^{1,2}$ -PEG gel, and t_0 is the thickness of the corresponding as-prepared Tetra-PEG gel. All measurements were performed in 3 times at 25 °C.

3.3 Results and Discussion

3.3-1 Distribution of molecular stents inside the St-PEG gels

In this study, quite dense molecular stents were introduced into the Tetra-PEG gels. For the localization of the molecular stents, all the St-PEG gels were transparent and homogeneous in appearance, suggesting no large aggregation in the gels. **Figure 3-3** shows the pictures of St²-PEG gels. Moreover, cross section of the St²-PEG gels dyed with methylene blue to stain PNaAMPS was uniform. Based on these results, the molecular stents distributed homogeneously within the St-PEG gels. However, at the molecular scale, the molecular stent distribution would not be perfectly homogeneous because of the interactions between the polymers.

3.3-2 Excessively swelling behavior of the St-PEG gels by multi-step molecular stent method

Figure 3-4 shows the swelling ratio of St²-PEG gels prepared by the multi-step molecular stent method. Regardless of the molecular weight of the prepolymers used for the Tetra-PEG gel preparation, a clear difference in swelling ratio was observed between St²-PEG gels repeatedly introduced with the ionic molecular stent (PNaAMPS) and those alternately introduced with the ionic (PNaAMPS) and nonionic (PAAm) molecular stents. The swelling ratio of the St²-PEG gels with PNaAMPS/PAAm was about 1.15 times higher (for the 10 k) and about 1.3 times higher (for the 20 k) than that of the gels with PNaAMPS/PNaAMPS. In the case of the Tetra-PEG gels (20 k), the prepolymer concentration in the preparation is lower than that of the gels (10 k), resulting in a lower elastic pressure arising from the network strands. In semi-dilute solutions, the osmotic pressure generated by molecular stents is determined by their concentration not their molecular weight.[6] Therefore, the osmotic pressure can be considered nearly identical if the same concentration of molecular stents is introduced into each gel. Based

on this assumption, the observed differences in swelling ratio are likely attributable to differences in the elastic pressure of the gels. It should also be noted that these measurements were performed after immersing the St²-PEG gels in water for 24 hours. Previous studies have shown that the leakage of molecular stents during this period is negligible.[7] Thus, the effect of stent leakage on the swelling ratio can be reasonably disregarded.

3.4 Conclusion of this chapter

Microscopic and dyeing observations confirmed that molecular stents were homogeneously distributed within the St-PEG gels, without visible aggregation. However, the interactions between the molecular stents and PEG at the microscopic scale remain unclear. Swelling experiments using the multi-step stent introduction method revealed that alternately introduction of ionic (PNaAMPS) and nonionic (PAAm) molecular stents induced greater swelling than using only ionic molecular stents.

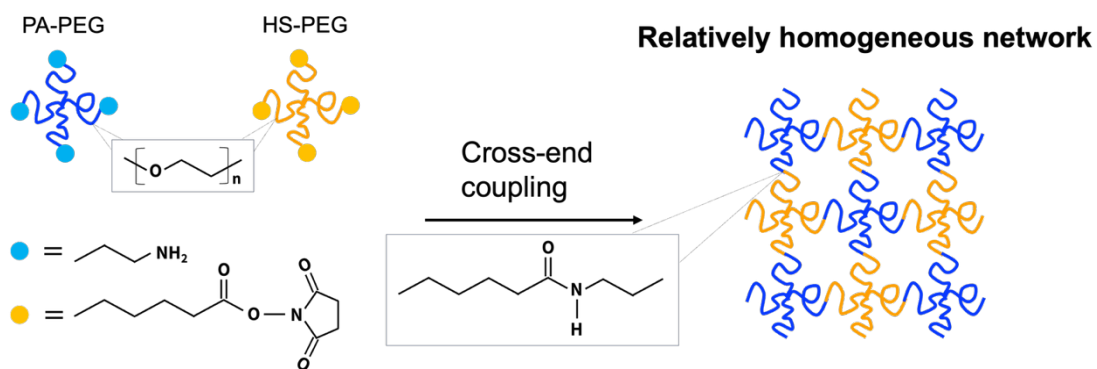


Figure 3-1. Illustration of the Tetra-PEG gel network in this work. Each prepolymers are crosslinked via amide bond formation. Figures Reproduced from *Small*, with permission from Wiley-VCH.

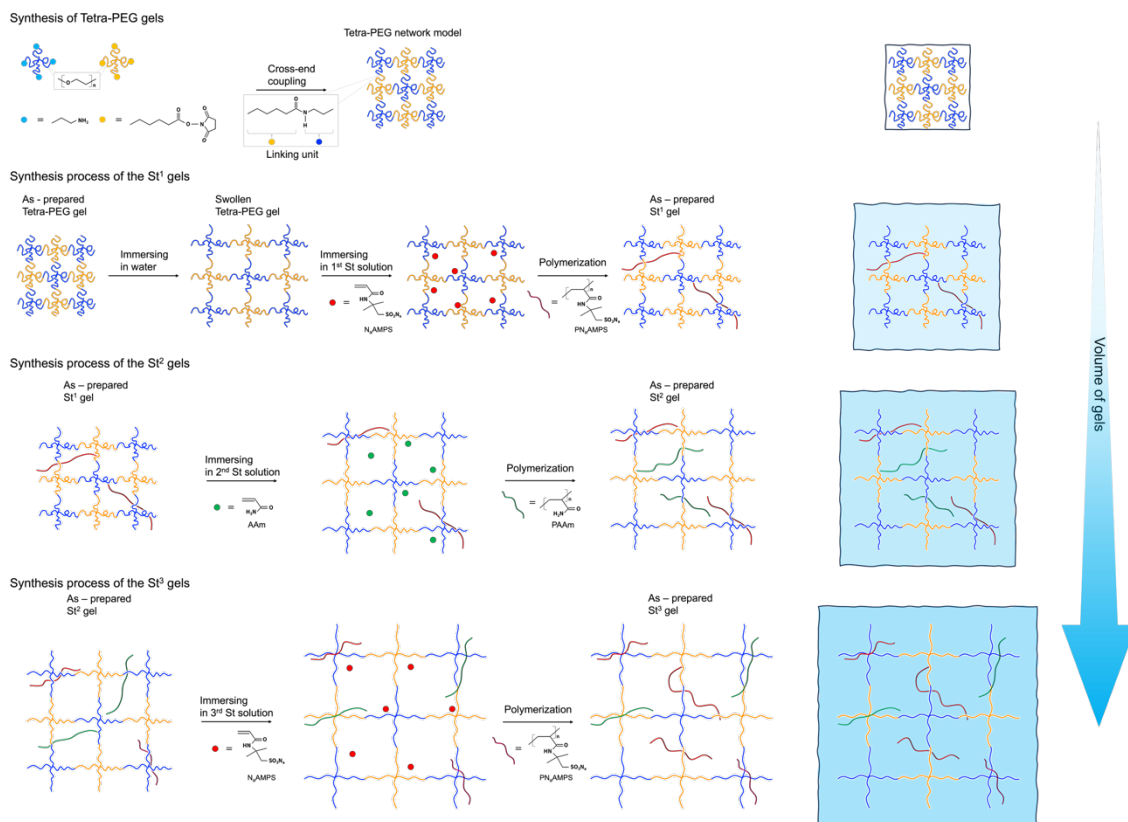


Figure 3-2. Schematic illustration of multi-step molecular stent method. Figures Reproduced from *Small*, with permission from Wiley-VCH.

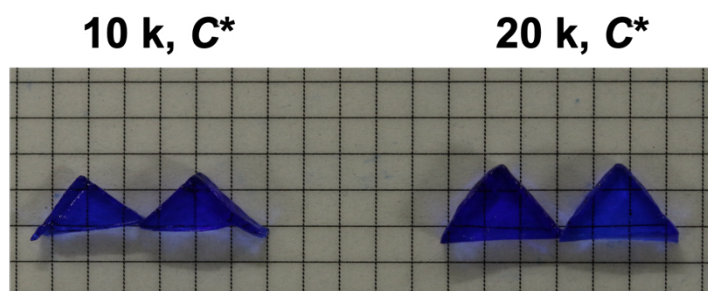


Figure 3-3. Picture of cross section of St²-PEG gels stained by Methylene blue.

Macroscopic observations showed that the stained PNaAMPS was homogeneously distributed throughout the gels. Figures Reproduced from *Small*, with permission from Wiley-VCH.

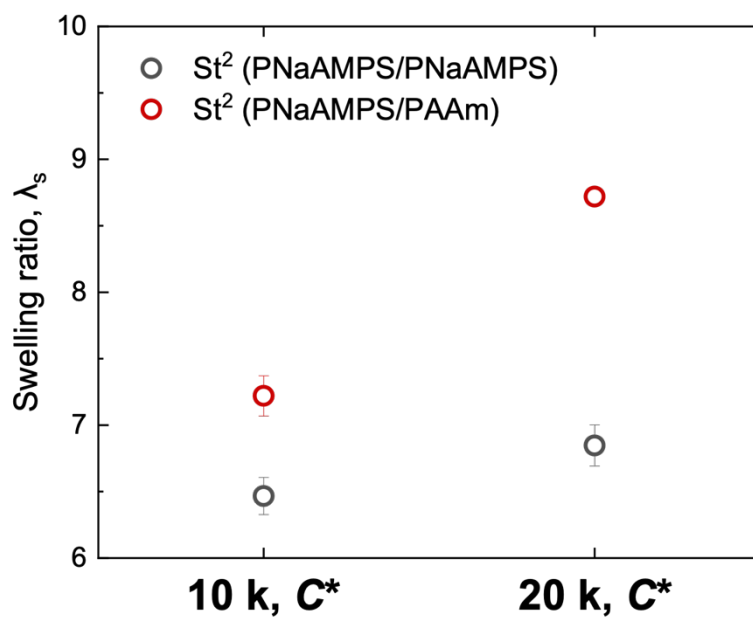


Figure 3-4. Swelling ratio of the swollen St²-PEG gels with different molecular stents.

The black circles correspond to the data of the swollen St²-PEG gels with PNaAMPS as the 1st and 2nd molecular stents, and the red circles correspond to those of the swollen St²-PEG gels with PNaAMPS as the 1st molecular stent and PAAm as the 2nd molecular stent. Prepolymers conditions of the Tetra-PEG gels in the fabrication process are 10 k, C*, 20 k, C*, respectively. Figures Reproduced from *Small*, with permission from Wiley-VCH.

3.5 References

1. Sakai, T., Matsunaga, T., Yamamoto, Y., Ito, C., Yoshida, R., Suzuki, S., Sasaki, N., Shibayama, M. and Chung, U.-I. "Design and Fabrication of a High-Stretch Hydrogel with Ideally Homogeneous Network Structure from Tetrahedron-like Macromonomers" *Macromolecules* **41**, 5379–5384 (2008).
2. Sakai, T. "Gelation mechanism and mechanical properties of Tetra-PEG gel" *React. Funct. Polym.* **73**, 898–903 (2013).
3. Akagi, Y., Katashima, T., Sakurai, H., Chung, U.-I. and Sakai, T. "Ultimate elongation of polymer gels with controlled network structure" *RSC Advances* **3**, 13251–13258 (2013).
4. Li, X., Khairulina, K., Chung, U. and Sakai, T. "Electrophoretic Mobility of Double-Stranded DNA in Polymer Solutions and Gels with Tuned Structures" *Macromolecules* **47**, 3582–3586 (2014).
5. Nishi, K., Fujii, K., Chung, U.-I., Shibayama, M. and Sakai, T. "Experimental Observation of Two Featured Unexpected from the Classical Theories of Rubber Elasticity" *Phys. Rev. Lett.* **119**, 267801-1–6 (2017).
6. Takamasa, Sakai "Physics of Polymer Gels" Ed.; Wiley-VCH: Weinheim, 2020.
7. Hoshino, K., Nakajima, T., Matsuda, T., Sakai, T. and Gong, J. P. "Network elasticity of a model hydrogel as a function of swelling ratio: from shrinking to extreme swelling state" *Soft matter* **14**, 9693–9701 (2018).

Chapter 4: Excess Swelling of the Tetra-PEG Gel Networks

4.1 Introduction

In the previous chapter, we confirmed that the multi-step molecular stent method—where molecular stents of different chemical species are introduced alternately—is an effective approach for inducing efficient and excessive swelling of the gels. In this chapter, we apply this method to excessively swelling of the Tetra-PEG gels, and discuss the resulting mechanical properties in relation to the stretching state of the network strands. In the following sections, we describe how to derive the structural swelling limit and the elastic energy stored per network strands, which are essential for the subsequent discussion.

4.1-1 Structural swelling limit

First, we explain the method for deriving the mean square end-to-end distance, R_0 , of the network strands in the as-prepared Tetra-PEG gel. The R_0 of the Tetra-PEG gels was calculated based on their prepolymer conditions.[1, 2] Under the affine deformation assumption where the macroscopic and microscopic deformations are well coupled, the theoretical maximum uniaxial swelling ratio, $\lambda_{\max}$, is considered to be estimated to $R_{\max}/R_0$, where R_0 and $R_{\max}$ are the mean square end-to-end distance of the as-prepared Tetra-PEG network strands and the average contour length of the strands, respectively. R_0 is estimated from the end-to-end distance of the Tetra-PEG gel prepared from the prepolymer solution at C^* . Since R_0 is not strongly dependent on the prepolymer concentration in the Tetra-PEG gel preparation, the following calculations were performed based on C^* , which is commonly used as the standard concentration in Tetra-PEG gel synthesis.[1, 3] At this overlapping concentration, the number density of prepolymers in the Tetra-PEG gel precursors, d , was calculated by the following equation;

$$d = \frac{C^* N_A}{M_w}. \quad (\text{Equation 15})$$

Here, N_A is Avogadro's constant. The value of C^* is 75 g L⁻¹ (for 10 k) and 40 g L⁻¹ (for 20 k). Assuming that prepolymers form a diamond-like lattice in the as-prepared Tetra-PEG gels^[18], the mean square end-to-end distance between the neighborhood cross-linking points in the as-prepared Tetra-PEG gels, R_0 , can be calculated by the same procedure as that for deriving the nearest-neighbor atomic distance. Thus, R_0 is written as the following equation;

$$R_0 = \frac{3}{2} d^{-\frac{1}{3}} = \frac{3}{2} \left(\frac{C^* N_A}{M_w} \right)^{-\frac{1}{3}}. \quad (\text{Equation 16})$$

Next, we explain the method for deriving the average contour length of the strands, $R_{\max}$. In aqueous environments, PEG networks typically adopt a *trans-trans-gauche* conformation.[4] However, as the network strands approach their extension limit, they transition to an all-*trans* conformation. In this all-*trans* conformation, the ethylene glycol units in the network strands align on a single plane. Also we assume that bond length and bond angle in the ethylene glycol does not change by extremely stretching, $R_{\max}$ can be written like the following equation:[2]

$$R_{\max} = N(r_1 + r_2) \sin\left(\frac{\theta}{2}\right). \quad (\text{Equation 17})$$

Here, r_1 and r_2 are the bond lengths of C-C (0.154 nm) and C-O (0.143 nm), and θ is the bond angle (109.5°), respectively, thus the length of ethylene glycol per unit is 0.36 nm. N is the average polymerization degree of the PEG strands (112 for 10k and 224 for 20k).[5]

Calculated R_0 are 4.44 (10 k), 8.14 (20 k), and $R_{\max}$ are 41 (10 k), 81.9 (20 k), so each $\lambda_{\max}$ are 9.2 and 10.1, respectively (**Figure 4-1**).

4.1-2 Calculation of elastic energy per strand from the elastic modulus of the gels

From the modulus measurements, the elastic energy density of the gel network was estimated by extending the works of Panyukov *et al.*[6, 7] The scaling relationship for the elastic energy density, W , and the elastic modulus, E , of a gel network is written as a function of the swelling ratio λ_s as

$$W \cong E \cong \nu_e k T \lambda_s^2 \quad (\text{Equation 18})$$

where ν_e is elastically effective network strand density, k is Boltzmann constant (1.38×10^{-21} J K⁻¹), and T is absolute temperature (298 K), so the resulted value of kT is about 4.0×10^{-19} J. This equation suggests that the elastic energy per strand, $U \cong W/\nu_e$, is around $kT \times \lambda_s^2$. The elastic energy per a network strand in the as-prepared Tetra-PEG gels, U_0 , is given by

$$U_0 \cong kT. \quad (\text{Equation 19})$$

Also, W can be written like the following equation:

$$W \cong \nu_e \times U. \quad (\text{Equation 20})$$

From Equation 18 and 19, the elastic modulus, E , can be roughly expressed as follows:

$$E \approx \nu_e \times U. \quad (\text{Equation 21})$$

Here, Equation 18 is valid only in the small swelling regime where the network strands can be regarded as Hookean springs and their elastic energy is proportional to the square of the swelling ratio λ_s . Considering the large swelling regime where the network strands are stretched extremely and the strands cannot be regarded as Hookean springs, Equation 18 can be generalized as follows:

$$W \cong E \cong \nu_e U(\lambda_s). \quad (\text{Equation 22})$$

Equation 19 and 22 leads the following relationship,

$$\frac{U}{U_0} \approx \frac{E \nu_{e,0}}{E_0 \nu_e} = \frac{E \lambda_s^3}{E_0}, \quad (\text{Equation 23})$$

where $\nu_{e,0}$ is ν_e of an as-prepared gel with the relationship of $\nu_e = \nu_{e,0} \lambda_s^{-3}$.

Substitution of Equation 19 into Equation 23 leads the following equation for rough estimation

of U as

$$U \approx \frac{E\lambda_s^3 kT}{E_0}. \quad (\text{Equation 24})$$

4.2 Experimental-Materials and Methods-

4.2-1 Indentation test of the gels

The indentation test was conducted to determine the Young's modulus of the gels using a universal mechanical tester (Shimadzu Autograph AGX-V, Shimadzu Co.). A hemispherical steel indenter (diameter: 0.5 mm) attached to a 10 N load cell was indented into the gel at a velocity of 0.5 mm min⁻¹. This indentation speed was sufficiently low to neglect viscoelastic effects, yet high enough to prevent water oozing from the gel surface near the indentation area.[8] The Young's modulus, E , was calculated based on the following form of Hertzian contact theory, which is applicable to soft materials under small deformation conditions: [9–11]

$$E = \frac{3f(1 - \nu_p^2)}{4l^{\frac{3}{2}}R^{\frac{1}{2}}}, \quad (\text{Equation 25})$$

where l is the indentation depth, R is the radius of the indenter (0.5 mm), f is the applied force, and ν_p is the Poisson's ratio, assumed to be 0.5. The modulus E was determined from the f - l relationship in the range $0 < l < 0.1$ mm. To eliminate any influence from the sample thickness, all gel specimens were prepared with thicknesses greater than 2 mm. All measurements were performed 4 times at 25 °C.

4.3 Results and Discussion

4.3-1 Excessive swelling behavior of the Tetra-PEG gel network

Figure 4-2 show photographs of the Tetra-PEG gels and St¹ and St²-PEG gels prepared under prepolymer conditions of (A) 10k, C*, and (B) 20k, C*, respectively. In both systems, the as-prepared Tetra-PEG gels were shaped into squares with dimensions of 0.5×0.5 cm². The Tetra-PEG gels without molecular stents showed minimal swelling in water. In contrast, the St¹-PEG gels which contain molecular stents showed excessively swelling beyond the original equilibrium swelling state of the Tetra-PEG gels. Furthermore, the St²-PEG gels which contain a higher dense molecular stents swelled even more than the St¹-PEG gels. This increase in equilibrium swelling ratio due to the introduction of molecular stents is attributed to the high osmotic pressure generated by the molecular stents. Similar excessive swelling behavior was observed for St-PEG gels regardless of their prepolymer conditions (**Figure 4-3**).

Figure 4-4 shows the uniaxial swelling ratio, λ_s , of the Tetra-PEG gels and the corresponding St-PEG gels. The dotted lines represent the estimated theoretical structural swelling limit, $\lambda_{\max}$, for each gel, based on the average end-to-end distance between neighboring crosslinking points in the as-prepared Tetra-PEG gels and the contour length of the corresponding PEG network strands (Section 4.1-1). The λ_s values of the Tetra-PEG gels without molecular stents were below 1.6, far from their structural swelling limits. In contrast, the St¹-PEG gels exhibited a clear increase in λ_s , and the St²-PEG gels showed a further increase. The λ_s values of both St²-PEG gels were close to their respective $\lambda_{\max}$, suggesting that the PEG network strands in the St²-PEG gels almost reached an extremely extended state. Similar excessive swelling was also observed in other Tetra-PEG gels prepared under different prepolymer conditions.

Moreover, the λ_s values of the 20 k St-PEG gels tend to be larger than those of the 10 k St-PEG gels (Figure 4-4). As explained above, the equilibrium swelling ratio of a gel is governed by the balance between osmotic pressure and elastic pressure. Elastic pressure generally increases with the density of the original network strands. In this study, the as-prepared 20 k Tetra-PEG gels had a lower network strand density and consequently lower elastic pressure than the 10 k gels. As a result, the 20 k St-PEG gels swelled more than the 10 k St-PEG gels. St²-PEG gels prepared by under C^* showed a little bit higher λ_s than each $\lambda_{\max}$. The Tetra-PEG gels prepared under conditions below C^* have internal defects in the gel network. As a result, the St-PEG gels synthesized based on these defective Tetra-PEG gels are thought to have swelled more than usual. Since the value of R_0 is largely independent of the Tetra-PEG gel preparation conditions (Section 4.1-1), the corresponding $\lambda_{\max}$ is also generally independent of those conditions. However, it should be noted that the presence of internal defects in the gel may alter the effective value of $\lambda_{\max}$.

4.3-2 Elastic modulus change and elastic energy per strand

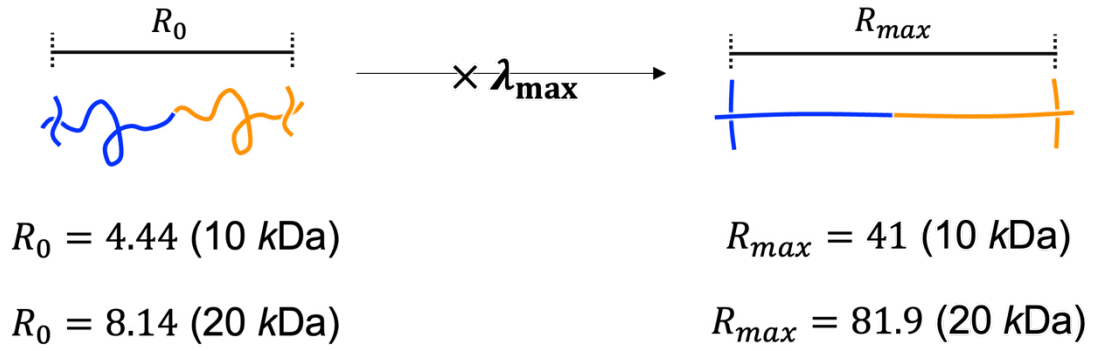
Young's modulus, E , of the gels was measured by indentation test to estimate the elastic energy stored in the network strands, U . **Figure 4-5** shows the relationship between the uniaxial swelling ratio and the normalized Young's modulus, E/E_0 , of swollen Tetra-PEG, St¹, and St²-PEG gels, where E_0 is the Young's modulus of the corresponding as-prepared Tetra-PEG gels. The dependence of E_0 on prepolymer concentration is shown in **Figure 4-6**. In all gels, E/E_0 decreased with increasing λ_s in the initial swelling regime, and then increased in the large swelling regime. Around $\lambda_{\max}$, E/E_0 of the St-PEG gels exceeded 1, even though the network strands were significantly diluted by swelling. A similar trend was observed for Tetra-PEG gels prepared under various prepolymer conditions (Figure 4-5).

Figure 4-7 shows the relationship between the uniaxial swelling ratio λ_s and the stored elastic energy U of the gels. When the deformation is sufficiently small, the end-to-end distance of the network strands follows a Gaussian distribution. In this regime, the strands behave elastically like Hookean springs, and U is proportional to the square of λ_s . [2] According to previous studies on Tetra-PEG gel swelling, a linear relationship between U and λ_s^2 is observed at low swelling regime ($\lambda_s < 3$), while nonlinear behavior appears at large swelling regime ($3 < \lambda_s$). As shown in Figure 4-7, the elastic energy, U of both St-PEG gels increased significantly and deviated markedly from the $U \propto \lambda_s^2$ relationship (Equation 18) in the large swelling regime where λ_s approaches $\lambda_{\max}$. This suggests that the extremely stretched PEG network strands no longer follow a Gaussian distribution and exhibit stiff, non-Hookean behavior. Based on equation 22, these results strongly indicate that the increase in E/E_0 at high λ_s is due to the dominant effect of highly elongated network strands overcoming the dilution effect (**Figure 4-8**). In contrast, the decrease in E/E_0 at low λ_s is attributed to network dilution with swelling. Regardless of the molecular weight (Mw) of the prepolymers, the St²-PEG gels prepared at the lowest prepolymer concentrations exhibited the highest elastic energy: $6.0 \times 10^3 \text{ kJ mol}^{-1}$ (10 k, 0.86 C*) and $3.0 \times 10^4 \text{ kJ mol}^{-1}$ (20 k, 0.75 C*) (Figure 4-7). This is because the PEG network strands in these gels are stretched to a greater extent due to their larger swelling.

4.4 Conclusion of this chapter

The introduction of molecular stents into the Tetra-PEG gels significantly enhanced their swelling capacity, allowing them to approach their structural swelling limits. The St²-PEG gels with a higher density of molecular stents exhibited the highest uniaxial swelling ratios, λ_s , particularly under low prepolymer concentrations due to their defects in the network structure. Elastic modulus measurements revealed a non-monotonic change with swelling: initial softening due to network dilution was followed by stiffening as network strands became highly extended. This was accompanied by a marked increase in stored elastic energy per strand, U , especially in the highly swollen St²-PEG gels. At large λ_s , deviation from Gaussian chain behavior indicated highly stretched network strands does not behave non-Hookean springs, with U no longer proportional to λ_s^2 .

$$\text{Structural swelling limit, } \lambda_{\max} = \frac{R_{\max}}{R_0}$$



$$\lambda_{\max} \cong 9.2 \text{ (10 kDa), } 10.1 \text{ (20 kDa)}$$

Figure 4-1. Illustration of the structural swelling limit. PEG network strands reaches their extension limit by stretching approximately 9.2 (10 k) and 10.1 (20 k), respectively.

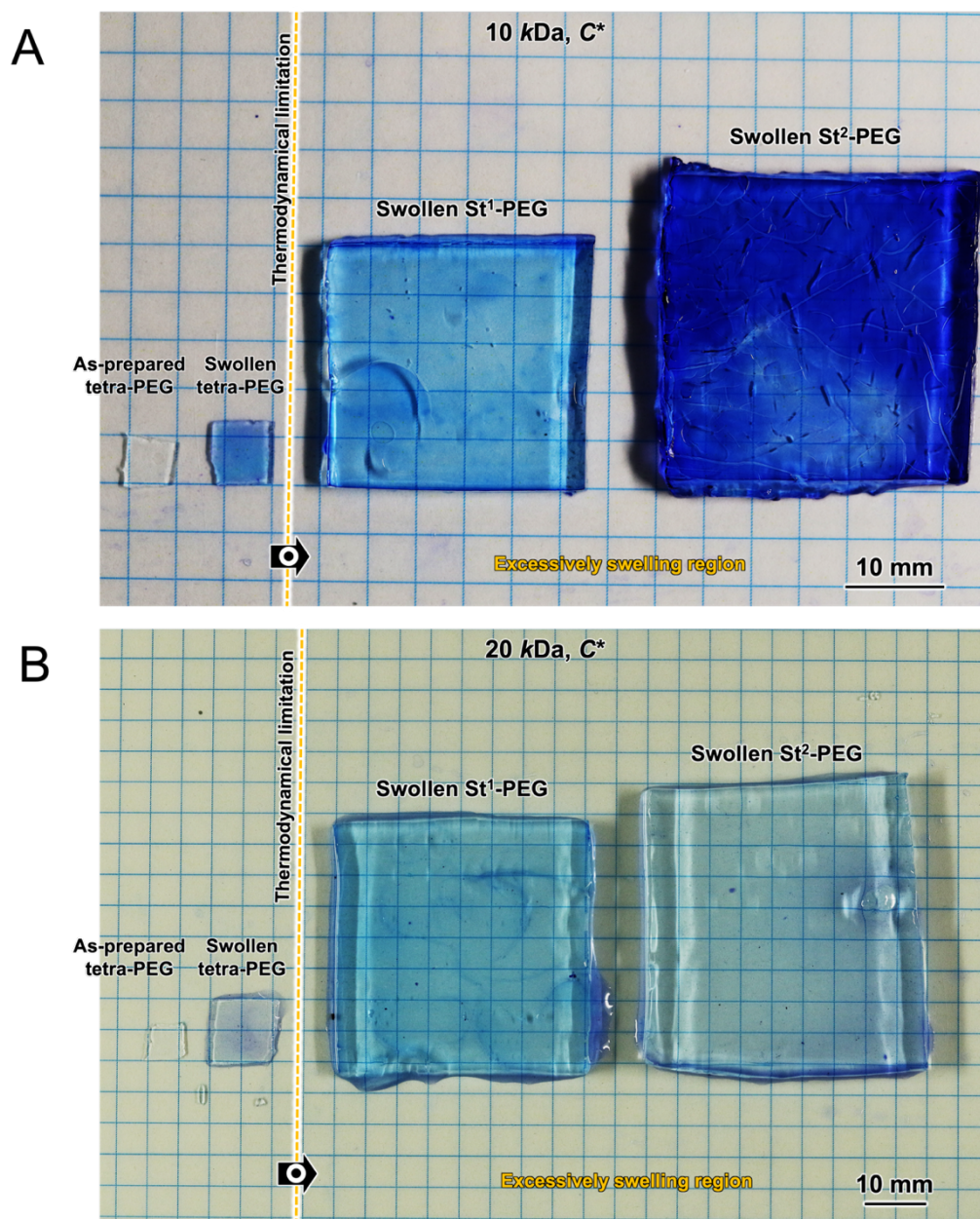


Figure 4-2. Pictures of the Tetra-PEG gels and corresponding St gels, where the prepolymer condition was (A) 10 k, C^* and (B) 20 k, C^* . From the left is as-prepared Tetra-PEG, swollen Tetra-PEG, St¹, St²-PEG gels. The swollen gels were stained with methylene blue for visualization. Figures Reproduced from *Small*, with permission from Wiley-VCH.

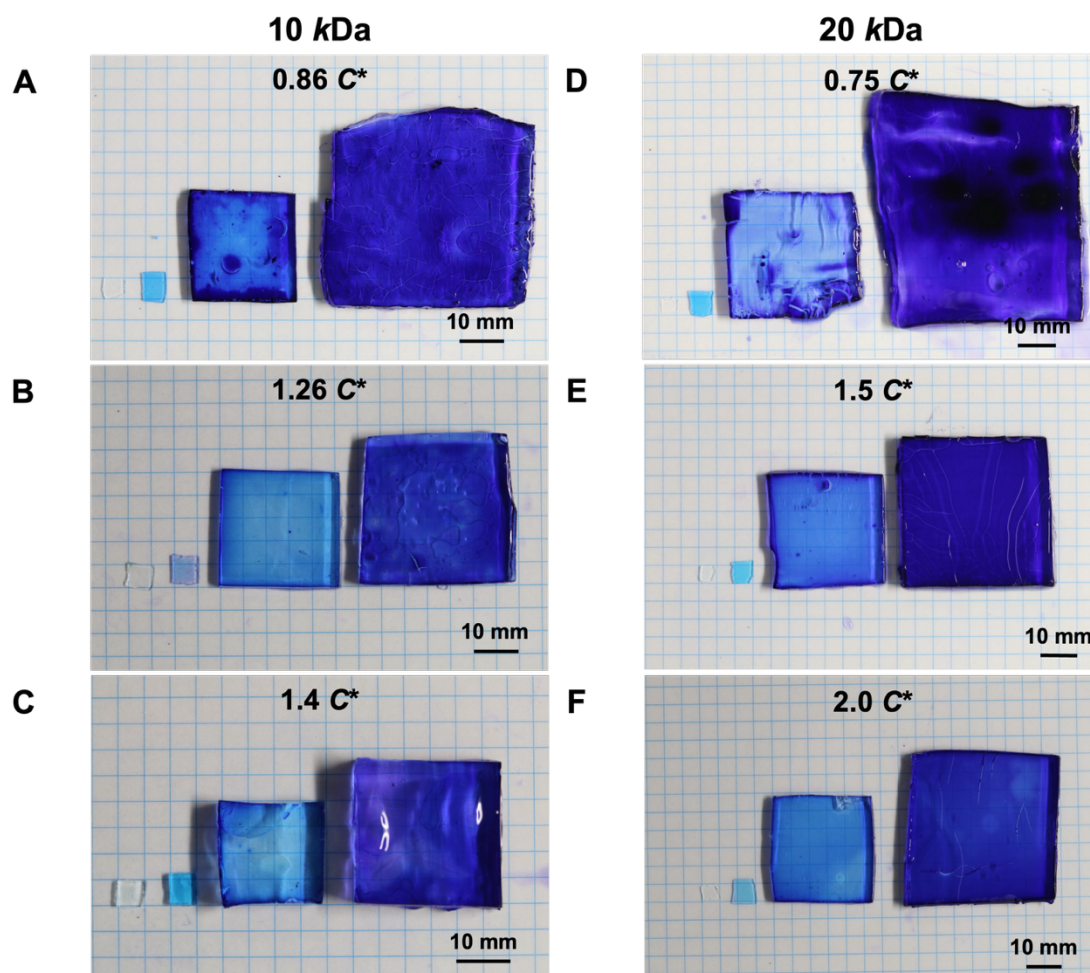


Figure 4-3. Swelling behavior of the Tetra-PEG gels and the corresponding St gels. (A–C) M_w :10 k, (A) $0.86 C^*$, (B) $1.26 C^*$, (C) $1.4 C^*$, and (D–F) M_w :20 k, (D) $0.75 C^*$, (E) $1.5 C^*$, (F) $2.0 C^*$. From the left is as-prepared Tetra-PEG, swollen Tetra-PEG, St^1 , and St^2 gels. Regardless of prepolymers conditions, the St gels showed excess swelling behavior. Also, λ_s of the St^2 -PEG gels increased with the decrease of prepolymer concentration. Figures Reproduced from *Small*, with permission from Wiley-VCH.

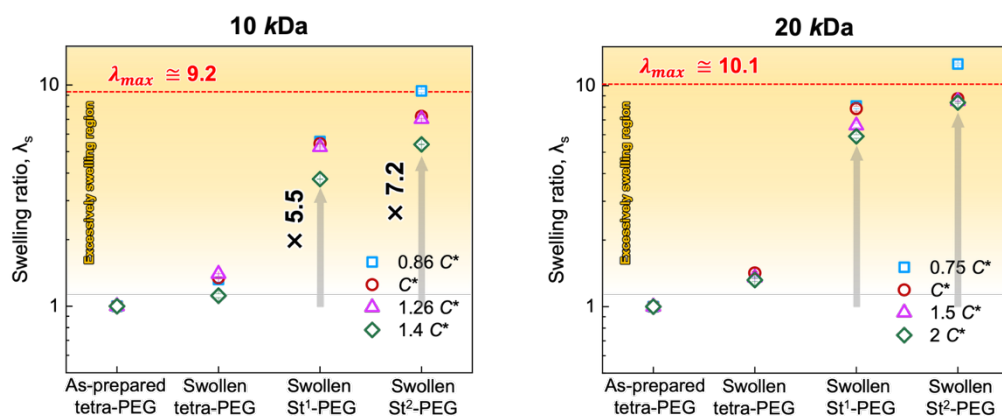


Figure 4-4. Uniaxial swelling ratio, λ_s , of the Tetra-PEG gels and corresponding St¹ and St²-PEG gels for various prepolymer conditions. The term “as-pre” stands for the as-prepared state. Black and red dotted lines show the theoretical uniaxial swelling limit, λ_{max} , based on C^* for 10 k and 20 k, respectively. Figures Reproduced from *Small*, with permission from Wiley-VCH.

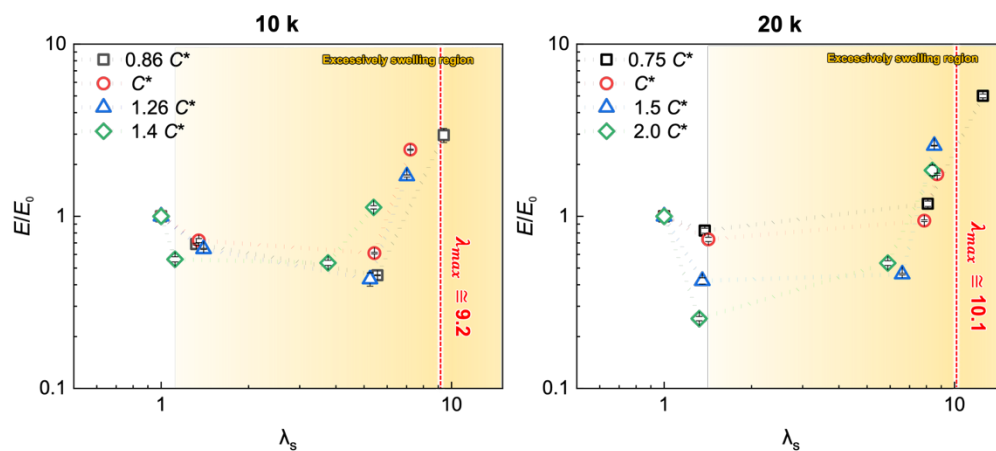


Figure 4-5. Normalized elastic modulus, E/E_0 , of the Tetra-PEG and St gels as a function of their uniaxial swelling ratio. The error bar represents mean $\pm$ SD ($N = 3$).

Figures Reproduced from *Small*, with permission from Wiley-VCH.

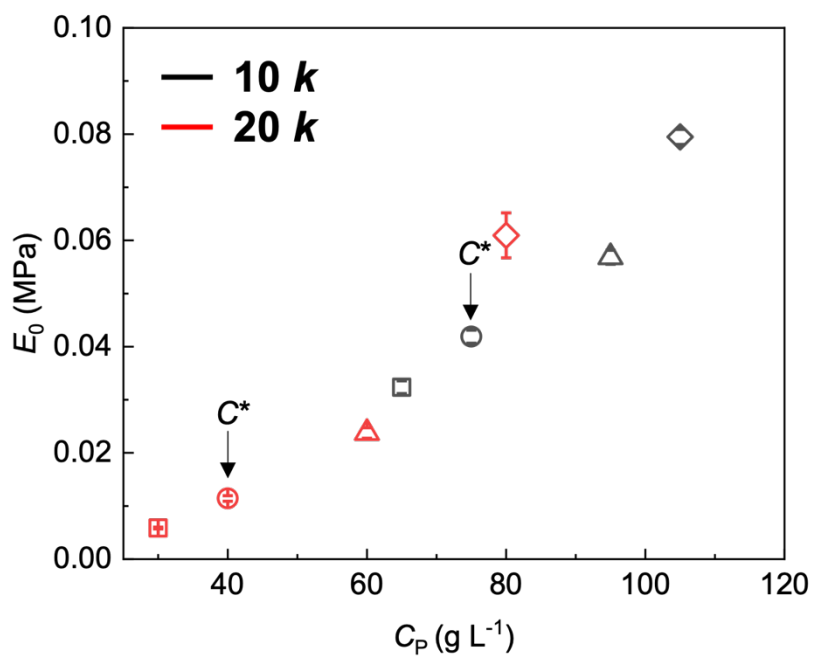


Figure 4-6. Elastic modulus of the as-prepared Tetra-PEG gels, E_0 , and their prepolymer concentration. E_0 of the as-prepared Tetra-PEG gels increased with increasing prepolymer concentration, regardless of molecular weight of prepolymers. Figures Reproduced from *Small*, with permission from Wiley-VCH.

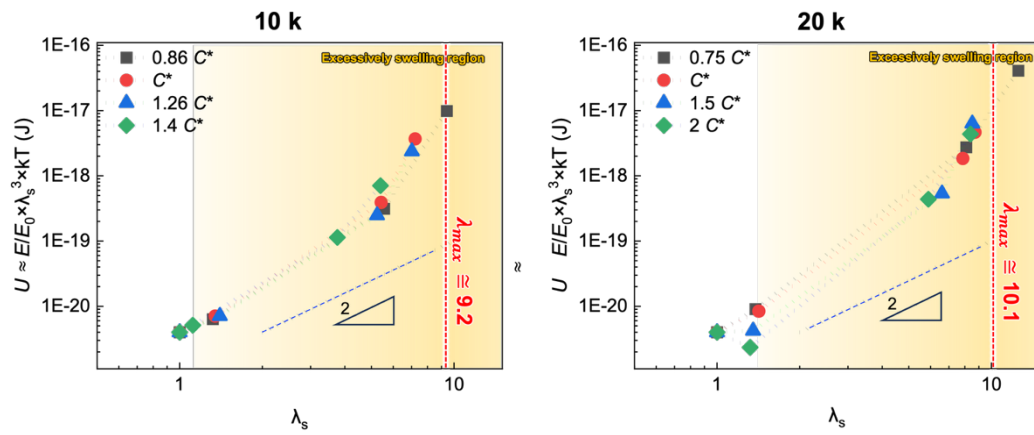


Figure 4-7. Relationship between uniaxial swelling ratio, λ_s , and stored elastic energy per a PEG network strand, U , of the Tetra-PEG gels and corresponding St gels. Blue dotted line shows the line with a slope of 2 ($U \propto \lambda_s^2$). Figures Reproduced from *Small*, with permission from Wiley-VCH.

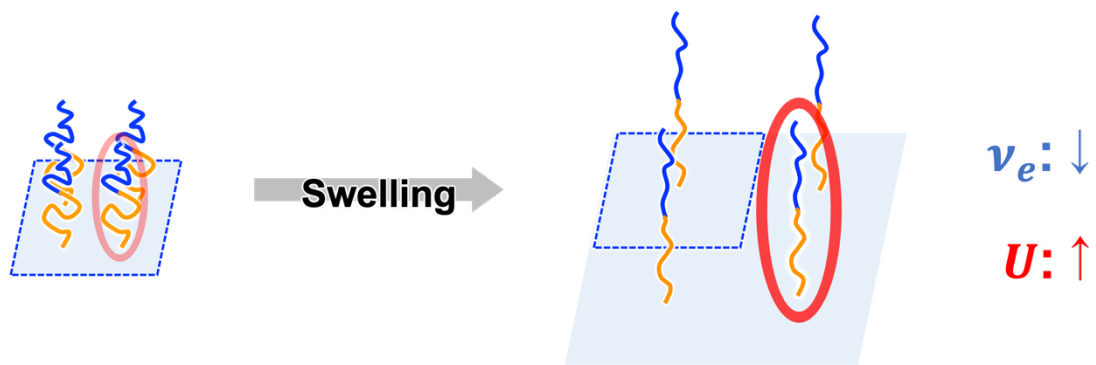


Figure 4-8. Elastic modulus can be separate into the contribution of ν_e and U based on Equation 21.

4.5 References

1. Takamasa Sakai “Physics of Polymer Gels” Ed.; Wiley-VCH: Weinheim, 2020.
2. Hoshino K., Nakajima T., Matsuda T., Sakai T. and Gong J. P. “Network elasticity of a model hydrogel as a function of swelling ratio: from shrinking to extreme swelling state” *Soft matter* **14**, 9693–9701 (2018).
3. Takahiro Matsuda, Naohiro Kashimura, Takamasa Sakai, Tasuku Nakajima, Takuto Matsushita and Jia Ping Gong “Yielding of Double-Network Hydrogels with Systematically Controlled Tetra-PEG First Networks” *Macromolecules* Accepted
4. Susanne Liese, Manuel Gensler, Stefanie Krysiak, Richard Schwarzl, Andreas Achazi, Beate Paulus, Thorsten Hugel, Jürgen P. Rabe and Roland R. Netz “Hydration Effects Turn a Highly Stretched Polymer from an Entropic into an Energetic Spring” *ACS Nano* **11**, 702–712 (2017).
5. Li X., Kondo S., Chung U.-I. and Sakai T. “Degradation Behavior of Polymer Gels Caused by Nonspecific Cleavages of Network Strands” *Chem. Mater.* **26**, 5352–5357 (2014).
6. Obukhov S. P., Rubinstein M. and Colby R. H. “Network Modulus and Superelasticity” *Macromolecules* **27**, 3191–3198 (1994).
7. Panyukov S. V. “Scaling theory of high elasticity” *Zh. Eksp. Teor. Fiz.* **98**, 668–680 (1990).
8. Hu Y., Zhao X., Vlassak J. J., Suo Z. Using indentation to characterize the poroelasticity of gels. *Appl. Phys. Lett.* **96**, 121904-1–3 (2010).
9. McKee C. T., Last J. A., Russell P., Murphy C. J. Indentation versus tensile measurement of Young’s modulus for soft biological tissues. *Tissue Eng. Part B Rev.* **17**, 155–164 (2011).

10. Lin D. C., Schreiber D. I., Dimitriadis E. K., Horkay F. Spherical indentation of soft matter beyond the Hertzian regime: numerical and experimental validation of hyperelastic models. *Biomech. Model. Mechanobiol.* **8**, 345–358 (2009).
11. Nathan R. Richbourg, Manuel K. Rausch and Nicholas A. Peppas “Cross-evaluation of stiffness measurement methods for hydrogels” *Polymer* **258**, 125316 (2022).

Chapter 5: *Osmotic Explosion (Bulk Disintegration) of the gels*

5.1 Introduction

In the previous chapter, the stiffening of Tetra-PEG gels with swelling was discussed in relation to the stretching ratio of the network strands. It was found that the network strands within the swollen St^2 -PEG gels were stretched to approximately the structural swelling limit, $\lambda_{\max}$. Therefore, when the St^3 -PEG gels are immersed in water, they are expected to swell beyond the structural limit. In this chapter, we describe the behavior of the St^3 -PEG gels when they swell beyond the structural swelling limit.

5.1-1 Calculation method of each polymer volume fraction

To estimate osmotic pressure generated by PEG networks, PNaAMPS (1st and 3rd molecular stent), and PAAm (2nd molecular stent), each polymer volume fraction, ϕ , of the gels was calculated. The detail method to calculate polymer volume fraction is described as follows.

As basic data, we measured the volume swelling ratio Q ($Q = \lambda_s^3$), total polymer weight fraction W^{polymer} , and density ρ of all gels. Superscripts and subscripts on these variables denote the target component and the state of the gel, respectively. We also used literature values for the density of each component (for polymers, the density of the dried form): $\rho^{\text{PNaAMPS}} = 1.739 \pm 0.3$, and $\rho^{\text{PAAm}} = 1.437 \pm 0.2$, and $\rho^{\text{PEG}} = 1.12 \text{ g cm}^{-3}$. [1, 2] We first estimated the weight fraction of each component and then converted it to the corresponding volume fraction. For the swollen Tetra-PEG gel, which is a PEG/water binary component system, the total polymer weight fraction equals the PEG weight fraction:[3]

$$W_{\text{swollen Tetra-PEG gels}}^{\text{PEG}} = W_{\text{swollen Tetra-PEG gels}}^{\text{polymer}} \quad (\text{Equation 26})$$

Based on this, the volume fraction, $\phi_{\text{swollen Tetra-PEG gels}}^{\text{PEG}}$ was calculated as

$$\phi_{\text{swollen Tetra-PEG gels}}^{\text{PEG}} = \frac{\frac{W_{\text{swollen Tetra-PEG gels}}^{\text{PEG}}}{\rho^{\text{polymer}}}}{\frac{W_{\text{swollen Tetra-PEG gels}}^{\text{PEG}}}{\rho^{\text{PEG}}} + \frac{1 - W_{\text{swollen Tetra-PEG gels}}^{\text{PEG}}}{\rho^{\text{water}}}}. \quad (\text{Equation 27})$$

The St¹-PEG gels is a PEG/PNaAMPS/water ternary component system, formed by introducing PNaAMPS into the swollen Tetra-PEG gel. Throughout this process, the total mass of the Tetra-PEG network in the swollen St-PEG gels remains unchanged. Thus, the weight fraction of Tetra-PEG in the swollen St¹-PEG gel, $W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}}$ is calculated as:

$$\begin{aligned} & W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}} \\ &= W_{\text{swollen Tetra-PEG gels}}^{\text{PEG}} \frac{\rho_{\text{swollen Tetra-PEG gels}} Q_{\text{swollen Tetra-PEG gels}}}{\rho_{\text{swollen St}^1\text{-PEG gels}} Q_{\text{swollen St}^1\text{-PEG gels}}}. \quad (\text{Equation 28}) \end{aligned}$$

Then, the weight fraction and volume fraction of PNaAMPS in the St¹-PEG gel ,

$W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}$ and $\phi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}$ are:

$$W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}} = W_{\text{swollen St}^1\text{-PEG gels}}^{\text{polymer}} - W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}}, \quad (\text{Equation 29})$$

$\phi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}$

$$= \frac{\frac{W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}}{\rho^{\text{PNaAMPS}}}}{\frac{W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}}}{\rho^{\text{PEG}}} + \frac{W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}}{\rho^{\text{PNaAMPS}}} + \frac{1 - W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}} - W_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}}}{\rho^{\text{water}}}}. \quad (\text{Equation 30})$$

The volume fraction of PEG in the swollen St¹-PEG gel, $\phi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}}$, was estimated from $\phi_{\text{swollen Tetra-PEG gels}}^{\text{PEG}}$ and their respective swelling ratio:

$$\begin{aligned} & \phi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}} \\ &= \phi_{\text{swollen Tetra-PEG gels}}^{\text{PEG}} \frac{Q_{\text{swollen Tetra-PEG gels}}}{Q_{\text{swollen St}^1\text{-PEG gels}}}. \quad (\text{Equation 31}) \end{aligned}$$

The volume fractions in St² and St³-PEG gels were calculated in a similar manner. For existing components, the volume fraction was updated from the previous step using the swelling ratio. For newly introduced components, the weight fraction was first determined by subtracting the known weight fractions of the other components, and then converted to volume fraction. As an example, for the swollen St²-PEG gels, the volume fractions of PEG and PNaAMPS were given by:

$$\varphi_{\text{swollen St}^2\text{-PEG gels}}^{\text{PEG}} = \varphi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PEG}} \frac{Q_{\text{swollen St}^1\text{-PEG gels}}}{Q_{\text{swollen St}^2\text{-PEG gels}}}, \quad (\text{Equation 32})$$

$$\varphi_{\text{swollen St}^2\text{-PEG gels}}^{\text{PNaAMPS}} = \varphi_{\text{swollen St}^1\text{-PEG gels}}^{\text{PNaAMPS}} \frac{Q_{\text{swollen St}^1\text{-PEG gels}}}{Q_{\text{swollen St}^2\text{-PEG gels}}}. \quad (\text{Equation 33})$$

The weight fraction of PAAm, a newly added component, was calculated as:

$$W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PAAm}} = W_{\text{swollen St}^2\text{-PEG gels}}^{\text{polymer}} - W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PEG}} - W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PNaAMPS}}, \quad (\text{Equation 34})$$

where $W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PEG}}$ and $W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PNaAMPS}}$ were estimated like Equation 28.

$\varphi_{\text{swollen St}^2\text{-PEG gels}}^{\text{PAAm}}$ was estimated from $W_{\text{swollen St}^2\text{-PEG gels}}^{\text{PAAm}}$ like Equation 30.

Finally, osmotic pressure of each gels was obtained by substituting these estimated volume fraction into Equation 9 and 10.

5.2 Experimental-Materials and Methods-

5.2-1 Measurement of Moisture Content and Density of Gels

The weight of the wet gel, w_{wet} , was measured using an electronic balance (XPE204, Mettler-Toledo International Inc.). The gel was then completely dried at 120 °C for 10 hours under vacuum conditions. The weight of the dried gel, w_{dry} , was measured using the same balance immediately after drying. The moisture content (W^{water}) and total polymer weight fraction (W^{polymer}) of the gels were calculated from the wet and dry weights as follows:

$$W^{\text{polymer}} = w_{\text{dry}} / w_{\text{wet}}, \quad (\text{Equation 35})$$

$$W^{\text{water}} = 1 - W^{\text{polymer}}. \quad (\text{Equation 36})$$

To determine the density of the gels, w_{wet} was measured both in air and in liquid paraffin (FUJIFILM Wako Pure Chemical Corporation) using an analytical balance (AUY220, Shimadzu Corporation). The density of the wet gel, denoted as ρ_{gel} , was calculated based on Archimedes' principle:

$$\rho_{\text{gel}} = \{w_{\text{wet,air}} / (w_{\text{wet,air}} - w_{\text{wet,liquid paraffin}})\} \times \rho^{\text{liquid paraffin}}. \quad (\text{Equation 37})$$

Here, $w_{\text{wet,air}}$ and $w_{\text{wet,liquid paraffin}}$ are the weights of the gels in the air and in the liquid paraffin, respectively, and $\rho^{\text{liquid paraffin}}$ is the density of the liquid paraffin (0.98 g cm⁻³). All measurements were performed 3 times at 25 °C .

5.2-2 Swelling test of the St³-PEG gels

As-prepared St³-PEG gels were cut into square shapes (10 × 10 mm²) using a razor blade. The cut gels were immersed in water at 25 °C and swelled for 24 hours. The swelling

behavior was monitored by capturing images every 30 seconds using a digital camera (EOS Kiss X5, Canon Inc.). After 24 hours, the swollen and degraded St³-PEG gels were gently agitated at approximately 1 Hz for another 24 hours using a shaker (PIC-100S, As One Co.). The resulting microgels were observed using the stereomicroscopic mode of a polarized optical microscope (ECLIPSE LV100 POL, Nikon Co.) with 2× and 5× objective lenses. Images of the microgels were captured using a digital camera (DS-Fi3, Nikon Co.).

5.2-3 Measurement of the Donnan Potential of St¹-PEG Gels

The Donnan potential of the gels was measured using the microelectrode technique. [4, 5] St¹-PEG gels were immersed in a 10⁻⁴ M NaCl solution, which served as the reference solution. A glass microelectrode (diameter: 150 nm) was used as the working electrode and inserted into the St¹-PEG gels at a speed of 2 μm/s using a micromanipulator (DMA-1511, NARISHIGE Group). A carbon reference electrode was placed in the reference solution. The potential difference between the working and reference electrodes was measured using an oscilloscope equipped with a preamplifier. All measurements were performed in 3 times at room temperature. Under the experimental condition of $C_g = 0.0213$ M, and Φ was measured as -96.5 mV with the microelectrode technique. From the above values and Equation 11, α was estimated as 0.201.

5.2-4 Fabrication of the PNaAMPS gels

The precursor solution for the PNaAMPS gels was prepared by dissolving 2 M 2-acrylamido-2-methylpropanesulfonic acid sodium salt (NaAMPS; Toa Gosei Co., Ltd.) and 2 mol% (to the monomer) N,N'-Methylenebis(acrylamide) (MBAA; Wako Pure Chemical Industries, Ltd.) as a crosslinker, 1 mol% (to the monomer) Ammonium persulfate (APS; Wako

Pure Chemical Industries, Ltd.) as a radical initiator, in water. To reduce the number of unreacted cross linking points, a relatively high concentration of APS was added. Then 0.1 mol% N,N,N',N'-Tetramethylethylenediamine (TEMED; Wako Pure Chemical Industries, Ltd.) was added in to the precursor solution. The final solution were injected into a glass mold. The rectangular glass mold was constructed using two glass plates and a silicone rubber spacer (0.5 or 3.0 mm thick) with an inner area of $80 \times 80 \text{ mm}^2$. The molds filled with the mixed prepolymer solution were sealed and left at 5°C for 24 hours to complete the reaction. Some of the resulting PNaAMPS gels were wrapped and stored at 5°C , while the rest were immersed in water for 24 hours to prepare equilibrium swollen PNaAMPS gels.

5.2-5 Fabrication of the St-PNaAMPS gels

The St-PNaAMPS gels were prepared by the introduction of nonionic molecular stents. The precursor solution for the nonionic stents (used for the 1st, 2nd, and 3rd molecular stents) was prepared by dissolving 3 M N,N-Dimethylacrylamide (DMAAm; Wako Pure Chemical Industries, Ltd.) and 0.5 mol% (to the monomer) α -keto in water.

To fabricate St¹-PNaAMPS gels, equilibrium swollen PNaAMPS gels were immersed in the nonionic precursor solution for 24 hours, picked up the gels from the solution, then sandwiched them between a pair of glass plates. The sandwiched gels were irradiated with UV light (peak wavelength: 365 nm) for 6 hours in a glove box filled with Ar gas to synthesize of linear PDMAAm (1st molecular stent) within the PNaAMPS gels.

To fabricate St²-PNaAMPS gels, the as-prepared St¹-PNaAMPS gels were immersed in the nonionic precursor solution for 24 hours, and PDMAAm (2nd molecular stent) was synthesized inside the St¹-PNaAMPS gels using the same UV irradiation procedure.

To fabricate St³-PNaAMPS gels, the as-prepared St²-PNaAMPS gels were again immersed in the nonionic precursor solution for 24 hours, and PDMAAm (3rd molecular stent) was synthesized within the St²-PNaAMPS gels using the same procedure.

The resulting St¹, St², and St³-PNaAMPS gels were stored at 5 °C in sealed plastic bags to prevent drying.

5.2-5 Cyclic swelling test of the St gels

PEG (M_w: 20 k, Wako Pure Chemical Industries, Ltd.) was dissolved in water to prepare PEG solutions with concentrations of 5, 10, and 20 wt.%. The St gels obtained from each gels were immersed in the corresponding PEG aqueous solutions and in pure water, corresponding to the swelling process. Subsequently, the equilibrium swollen St gels in pure water were immersed in the PEG aqueous solutions, corresponding to the deswelling process.

5.3 Results and Discussion

5.3-1 Bulk disintegration of the St³-PEG gels

Figure 4-3 showed that the St²-PEG gels nearly reached their structural swelling limit and stored significant elastic energy in their network strands. To investigate gels swelling beyond the structural swelling limit, St³-PEG gels were prepared by introducing an additional molecular stent into the St²-PEG gels and then immersing them in water. **Figure 5-1** show the swelling process of the St³-PEG gels (10 k, C* and 20 k, C*) in pure water. Interestingly, the St³-PEG gels began to disintegrate from the surface during swelling process. This observation suggests that excessively high osmotic pressure caused the gels to swell beyond their structural limits, leading to *osmotic explosion* of the bulk. After 24 hours of immersion, the disintegrated St³-PEG gels turned into slime-like body due to the entanglement of the linear molecular stents, which maintained the macroscopic integrity of the material (**Figure 5-2**). To fully disentangle the molecular stents and disperse the material, the degraded St³-PEG gels were gently shaken in water to produce a slurry.

Figure 5-3 show optical microscope images of the exploded St³-PEG gels (10 k, C* and 20 k, C*). Numerous microgel particles—fragments of the original St³-PEG gels—were observed within the slurry. The particle size and shape distributions were broad, with microgels ranging from sub-millimeter to several millimeters in size including other prepolymer conditions (**Figure 5-4**). Many of the microgels exhibited hierarchical surface cracks, suggesting a multi-scale fragmentation process regardless of the Tetra-PEG gel preparation method. The St³-PEG gels prepared under various conditions exhibited similar osmotic explosion behaviors, with the exception of certain gels such as those prepared under 10 k, 1.4 C* and 20 k, 0.75 C* conditions (Figure 5-2). In the case of the St³-PEG gels (10 k, 1.4 C*),

it is presumed that the osmotic pressure generated by the molecular stents was lower than the elastic pressure of the network strands. It prevented the swelling beyond its structural swelling limit and thus no osmotic explosion occurred. On the other hand, in the case of the St³-PEG gels (20 k, 0.75 C*), it is considered that severe leakage of the molecular stents occurred due to network defects, which halted the swelling before an osmotic explosion could take place. The osmotic explosion is reminiscent of osmotic hemolysis in red blood cells placed in hypotonic solutions, where excessive water uptake causes swelling and rupture of the cell membrane. While similar osmosis-induced ruptures are often observed in biological systems—for example, fruit splitting due to overwatering—the fragmentation of bulk 3D materials via osmotic forces has been rarely reported.

5.3-2 The criteria of the osmotic explosion

As mentioned above, the St³-PEG gels were eventually fragmented into microgels upon swelling. We hypothesize that this osmotic explosion of the Tetra-PEG gel networks—corresponding to the rupture of PEG network strands within the gels—occurs when the elastic energy per PEG network strand, denoted as U , exceeds the maximum elastic energy that a single PEG strand can withstand, denoted as $U_{\max}$ (**Figure 5-5**). According to the theory proposed by Lake and Thomas, $U_{\max}$ is given by the product of the bond dissociation energy of the weakest bond in the main chain, U_{BDE} , and the number of such bonds, n :^[6]

$$U_{\max} = U_{\text{BDE}} \times n. \quad (\text{Equation 38})$$

A PEG network strand in Tetra-PEG gels consists of repeating ethylene glycol units and a linking unit containing an alkyl chain and an amide bond. The bond dissociation energies of the covalent bonds in the main chain are as follows: 364 kJ mol⁻¹ (C–C bond), 331 kJ mol⁻¹ (C–O

bond), and 397 kJ mol⁻¹ (N–C bond in amide).[7, 8] Thus, the U_{BDE} for Tetra-PEG gels is 331 kJ mol⁻¹. As the main chain of a single ethylene glycol unit includes 3 covalent bonds and the linking unit includes 9 (**Figure 5-6**), the total U_{max} for a PEG strand can be estimated as:

$$(3 \times 331 \times n_{\text{PEG}} + 9 \times 331) \times N_{\text{A}}^{-1} \text{ kJ mol}^{-1}$$

Here, n is the average number of ethylene glycol units per strand (112 for 10 k, 224 for 20 k), and N_{A} is Avogadro's number. It should be noted that the actual activation energy required for strand rupture would be lower than this estimation, due to the assisting effect of stored elastic energy and the general decrease in activation energy with longer timescales.[9] Therefore, this estimation serves as an upper bound for U_{max} .

We then estimated the elastic energy per strand in the St³-PEG gels, U_{St^3} , and compared it to U_{max} . Although the St³-PEG gels do not reach a stable swelling equilibrium, we assumed that they would reach a virtual equilibrium at λ_{max} (the maximum swelling ratio) if no strand rupture occurred. Thus, using Equation 24, U_{St^3} was estimated as:

$$U_{\text{St}^3} = \frac{E_{\text{max}} \lambda_{\text{max}}^3 kT}{E_0}, \quad (\text{Equation 39})$$

where E_{max} is the elastic modulus of the St³-PEG gels at λ_{max} . Since E_{max} cannot be measured directly, we estimated it using osmotic pressure. At equilibrium swelling, the following relationship holds for isotropic gels:[10, 11]

$$\pi_{\text{mix}} + \pi_{\text{ion}} = -\pi_{\text{el}} \cong \frac{E}{3}. \quad (\text{Equation 40})$$

This equation allows the estimation of E from the osmotic pressure, which in turn can be calculated using the Flory–Huggins theory based on volume fractions. We first estimated the

osmotic pressure of the St³-PEG gels at $\lambda_{\max}$ and then used that to calculate $E_{\max}$. The estimated osmotic pressure of the gels are almost consistent with the ideal values (**Figure 5-7**). For the estimation of π_{mix} , the best fitting parameters for χ_{12} and χ_{13} in the Equation 13 are used to minimize the values of $(\pi_{\text{mix}} + \pi_{\text{ion}} + \pi_{\text{el}})^2$.

Figure 5-8 summarizes the estimated values of U and $U_{\max}$ for various prepolymer conditions. In cases where St³-PEG gels fragmented, the calculated U_{St^3} was close to or exceeded $U_{\max}$. Conversely, in the St³-PEG gels (10 k, 1.4 C*) that did not fragment, U_{St^3} remained at least one order of magnitude lower than $U_{\max}$.

Several factors may contribute to the observed hierarchical fragmentation. One is the distribution of polymerization degrees between crosslinking points. Although Tetra-PEG gels have relatively homogeneous networks, some distribution exists. Thus shorter strands are more likely to rupture first, forming cracks hierarchically from the surface. Another factor is the difference in swelling rates between the gel surface and interior. Since swelling begins at the surface, network strand elongation and breakage proceed from the outside inward, leading to finer cracks near the surface. Additionally, the oozing of molecular stents from fracture interfaces should be considered. As the stents are linear polymers, they gradually diffuse out from the gel surfaces and cracks. Although diffusion from bulk gels is negligible over experimental timescales, fragmentation shortens the diffusion distance, accelerating this process. When molecular stents ooze from the cracked surfaces, the local osmotic pressure drops, suppressing further nanoscale fracturing. Therefore, the St³-PEG gels fragmented only into microscale domains with surface cracks, rather than degrading to nanoscale (molecular) levels.

It is important to distinguish this phenomenon from previously reported swelling-induced crack propagation in gels.[12] That literature described macroscopic crack propagation due to stress localization at surface defects but did not observe bulk disintegration. In contrast, our study shows that excessive swelling applies considerable internal force throughout the entire gel network, leading to its disintegration into microgel fragments. In the case of St³-PEG gels (20 k, 0.75 C^*), U_{St^3} was significantly higher than U_{max} , yet fragmentation did not occur. Tetra-PEG networks prepared below C^* (including 20 k, 0.75 C^*) tend to have structural defects,[13] which may have facilitated premature leakage of molecular stents before sufficient osmotic pressure could develop—thereby suppressing fragmentation.

5.3-3 Excess swelling behavior of the PNaAMPS (heterogeneous) gel network

The PNaAMPS gel with a heterogeneous network structure was subjected to excessive swelling using a multi-step molecular stent method, and the resulting changes in its mechanical properties were evaluated. Furthermore, we examined the influence of network heterogeneity on the excessive swelling behavior and mechanical properties by comparing it with the Tetra-PEG gel. In this study, DMAAm was used as a nonionic monomer. Since DMAAm is both as a monomer and as a solvent, it is expected to allow a higher monomer concentration to be introduced into the PNaAMPS gels compared to when AAm is used.

Figure 5-9 shows the excessively swelling behavior of the PNaAMPS gel. It was found that the gel could excessively swell even with the introduction of only nonionic molecular stents, that is, by increasing only π_{mix} . However, the PNaAMPS gels did not disintegrate into microgels in a total of the 3 introduction steps unlike the case of Tetra-PEG gels.

Figure 5-10 shows the swelling ratio of the PNaAMPS gel. Since the PNaAMPS gel is composed of an ionic polymer, its swelling ratio at equilibrium is approximately 3, which is larger than that of the nonionic Tetra-PEG gel. With successive introduction of molecular stents, the swelling ratio further increased to approximately 5, 7, and 11, respectively.

As with the Tetra-PEG gels, the change in elastic modulus with swelling was also examined for the PNaAMPS gels. In the equilibrium swollen state, the PNaAMPS gels exhibited an elastic modulus ratio of approximately 2. This is attributed to the fact that, due to its intrinsic electrostatic repulsion and the contribution of π_{ion} , the network strands in PNaAMPS are already relatively stretched at equilibrium. In the region of $1 < \lambda_s < 3$, the elastic modulus must decreased and then increased, similar to the behavior observed in the Tetra-PEG gels. The St¹-PNaAMPS gels showed an exceptionally high elastic modulus, with a modulus ratio of approximately 15. However, at higher swelling ratio ($\lambda_s > 5$), the behavior diverged markedly from that of the Tetra-PEG gels. Specifically, the elastic moduli of the St² and St³-PNaAMPS gels decreased substantially, with modulus ratios of approximately 10 and 2, respectively.

Hereafter, this characteristic change in elastic modulus will be discussed based on the relationships described in Equations 21 and 23, in the same manner as the analysis of the Tetra-PEG gels. Based on the change in elastic modulus, we estimated the increase in elastic energy per single network strand (according to Equation 23). The results are shown in **Figure 5-11**. As discussed in the previous section, in the case of Tetra-PEG gels, the elastic energy increased and ultimately led to gel fragmentation. In contrast, for PNaAMPS gels, the elastic energy continued to increase even in the St²-PNaAMPS gels where a decrease in elastic modulus was observed. Also in the St³-PNaAMPS gels, the elastic energy was still maintained,

where the modulus further decreased,. Furthermore, no fragmentation was observed in the PNaAMPS gels. These differences are likely attributed to the heterogeneity of the network structure, which will be discussed in the following section.

5.3-4 Heterogeneity-dependent fracture of the network strands

The differences in the changes in elastic modulus and elastic energy with swelling between the Tetra-PEG gels and PNaAMPS gels were attributed to the heterogeneity-dependent rupture of network strands.[14] In this section, we discuss how the rupture behavior of network strands with swelling is affected by network heterogeneity, based on the results of cyclic swelling tests. Specifically, both St gels were immersed in PEG aqueous solutions of varying concentrations to induce swelling, and their elastic modulus were measured. Subsequently, the swollen St gels in water were transferred to PEG aqueous solutions of different concentrations to induce deswelling, followed by another measurement of the elastic modulus. By dissolving PEG into the external solvent, the external osmotic pressure, π_{external} , was increased, thereby controlling the swelling ratio of the St gels.

Figure 5-12 shows the modulus of the St gels with swelling process and deswelling process. In the case of the St¹-PEG gels, there was little difference in the elastic modulus between the swelling and deswelling processes, indicating that almost no network strand breakage occurred during the swelling process ($\lambda < \lambda_{\text{max}}$). For the St²-PEG gels, a slight difference in the elastic modulus was observed between the two processes, but the difference remained small. These results suggested that no significant network strand breakage occurs during the swelling process below λ_{max} due to the uniformity of the Tetra-PEG gels. In contrast, for the St¹ and St²-PNaAMPS gels, a significant decrease in elastic modulus was observed between the swelling and deswelling processes, which indicates that substantial

breakage of the network strands occurred during each swelling cycle. This observation reflects the structural inhomogeneity of the PNaAMPS gel networks.

From these results, it can be concluded that most network strands uniformly reach and exceed their extension limit around the structural swelling limit, leading to gel fragmentation in Tetra-PEG gels. On the other hand, in the PNaAMPS gels, relatively short network strands break first, followed by the gradual extension of longer strands with larger polymerization degree. This stepwise extension of network strands likely prevents gel fragmentation. Furthermore, the sustained high elastic energy can be explained by the newly extended long network strands accumulating elastic energy after the short network strands have broken.

5.4 Conclusion of this chapter

This study revealed that the introduction of molecular stents into Tetra-PEG gels 3 times induced excessive swelling beyond their structural swelling limit, leading to bulk disintegration into microgels—a phenomenon termed “*osmotic explosion*.” This rupture was caused because the elastic energy per strand exceeded the maximum energy that single PEG chain can withstand. In contrast, structurally heterogeneous PNaAMPS gels, despite undergoing similar osmotic stress, did not fragment. Cyclic swelling tests demonstrated that network strand rupture occurred uniformly in the homogeneous Tetra-PEG gels, while in heterogeneous PNaAMPS gels, rupture progressed from short to long network strands. This heterogeneity-dependent rupture suppressed catastrophic fragmentation and allowed sustained elastic energy accumulation in extended strands.

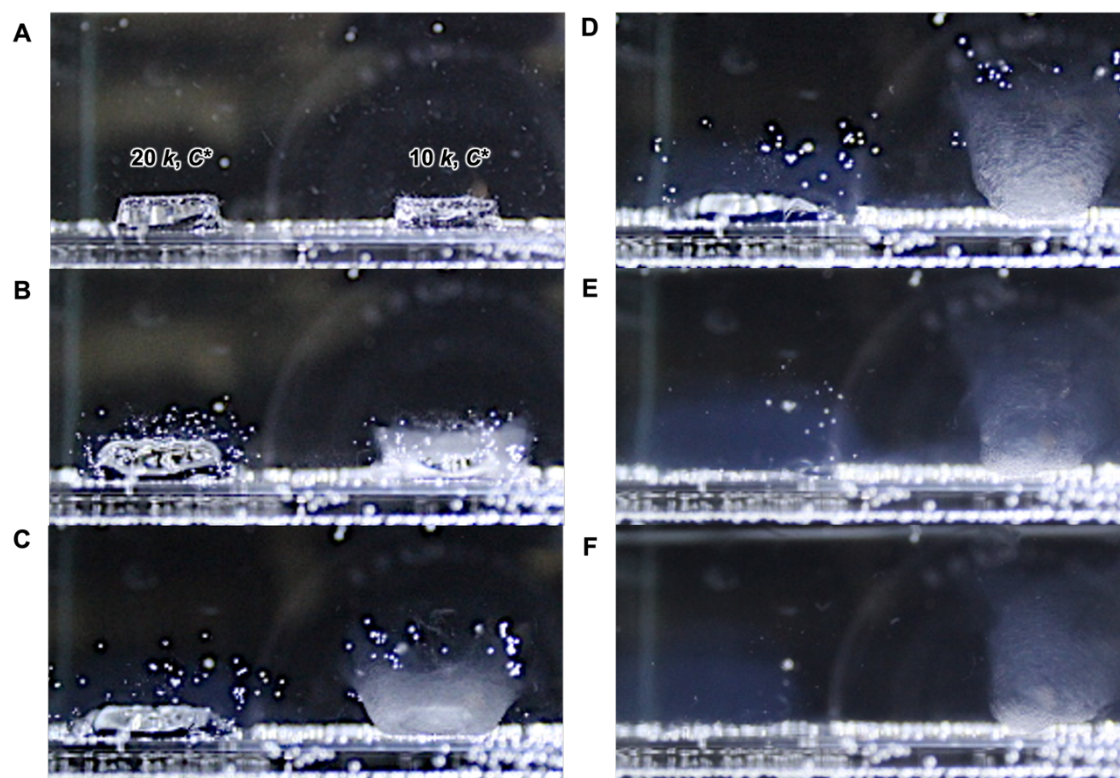


Figure 5-1. Osmotic explosion of the St³ gels in water. Pictures of the St³-PEG gels disintegrating in water. The time on each picture corresponds to the elapsed time after the immersion in water. Figures Reproduced from *Small*, with permission from Wiley-VCH.

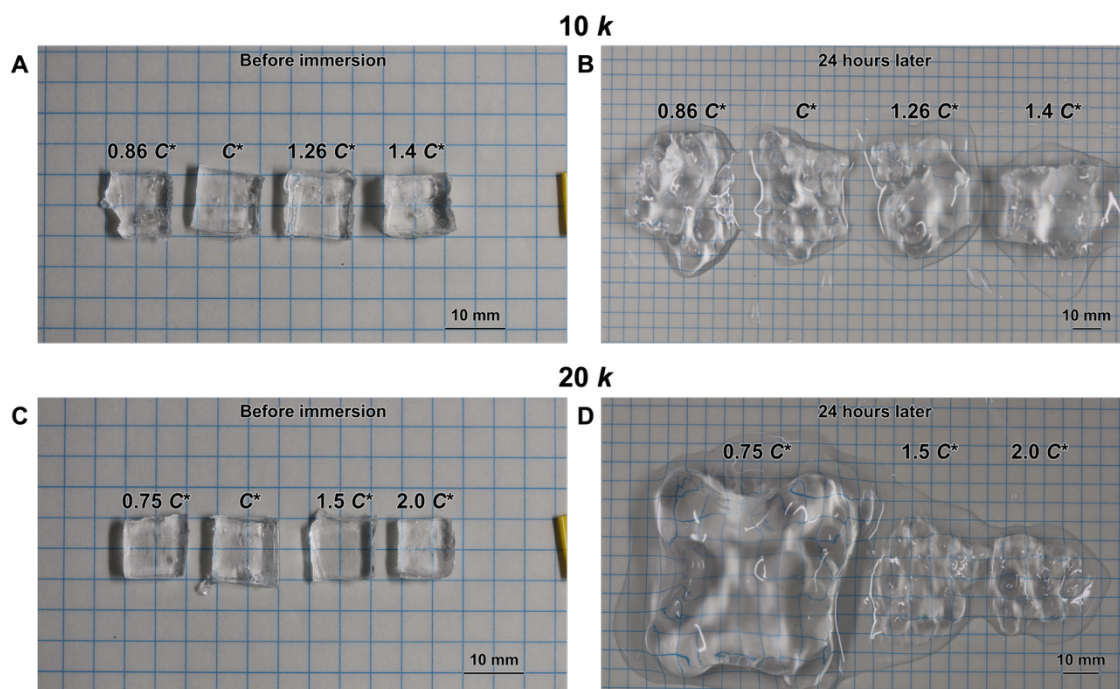


Figure 5-2. Pictures of the St³-PEG gels before and after immersion in water. (A, B) 10 k, (C, D) 20 k and (A, C) before immersion, (B, D) 24 hours later. The picture of the swollen St³-PEG gels (20 k, C*) could not be taken because of the handling problem of the gel. The entanglement of oozed molecular stents suppresses the spontaneous fragmentation of the St³-PEG gels. Figures Reproduced from *Small*, with permission from Wiley-VCH.

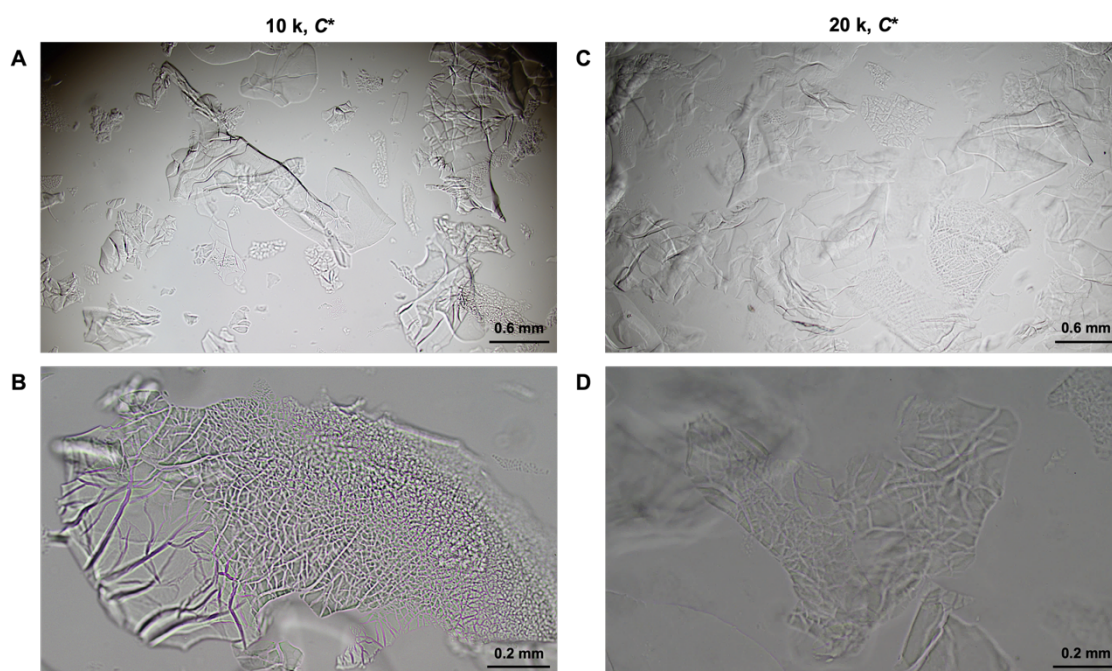


Figure 5-3. Optical microscope images of the microgels from St³-PEG gels: (A, B) 10 k, C* and (C, D) 20 k, C*. Figures Reproduced from *Small*, with permission from Wiley-VCH.

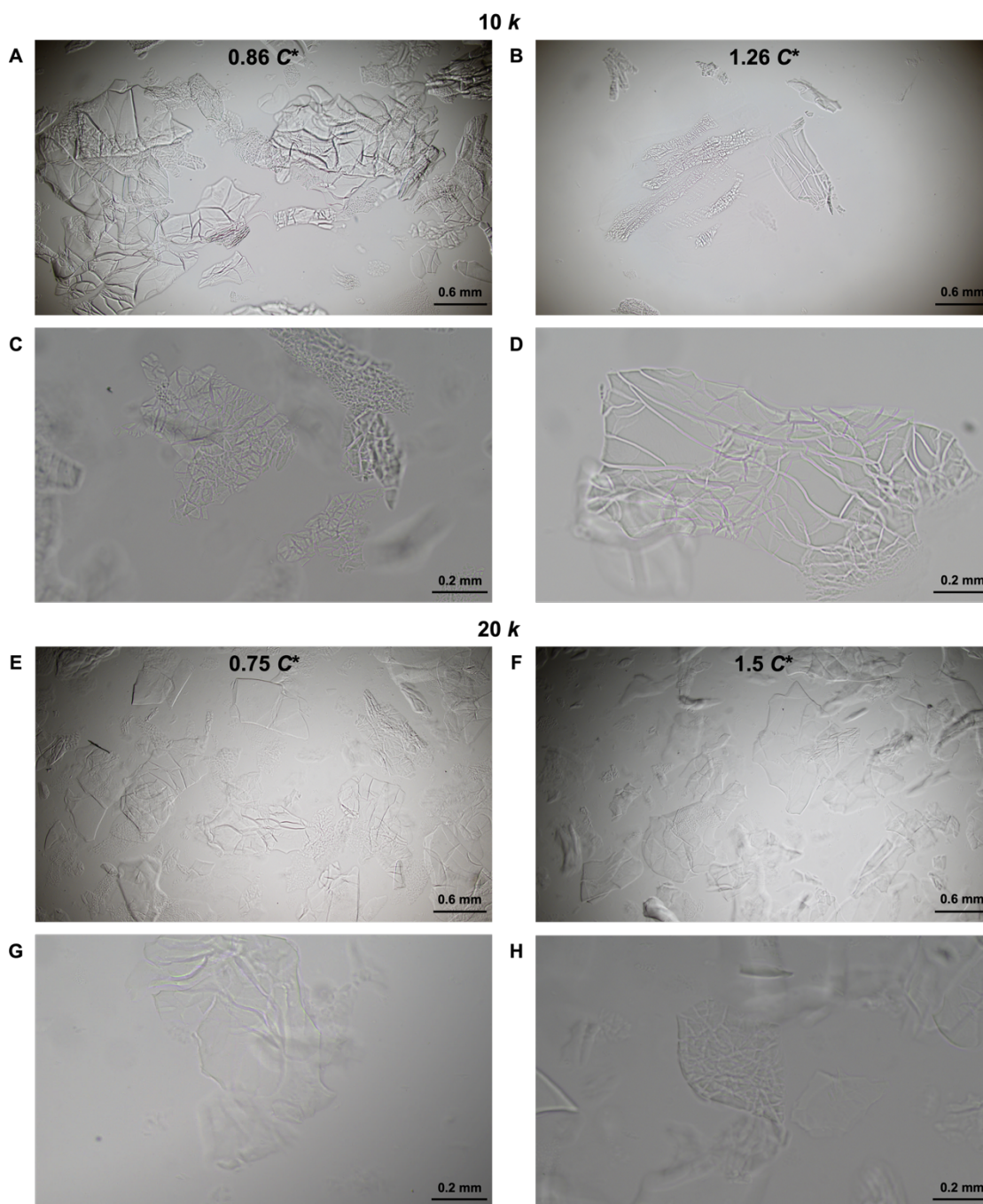


Figure 5-4. Microgels as the disintegrated products of the St³-PEG gels. (A, B, E, F) Low magnification, (C, D, G, H) high magnification pictures, (A–D) 10 k, and (E–H) 20 k. Prepolymer concentration is noted at the top of each figure. Figures Reproduced from *Small*, with permission from Wiley-VCH.

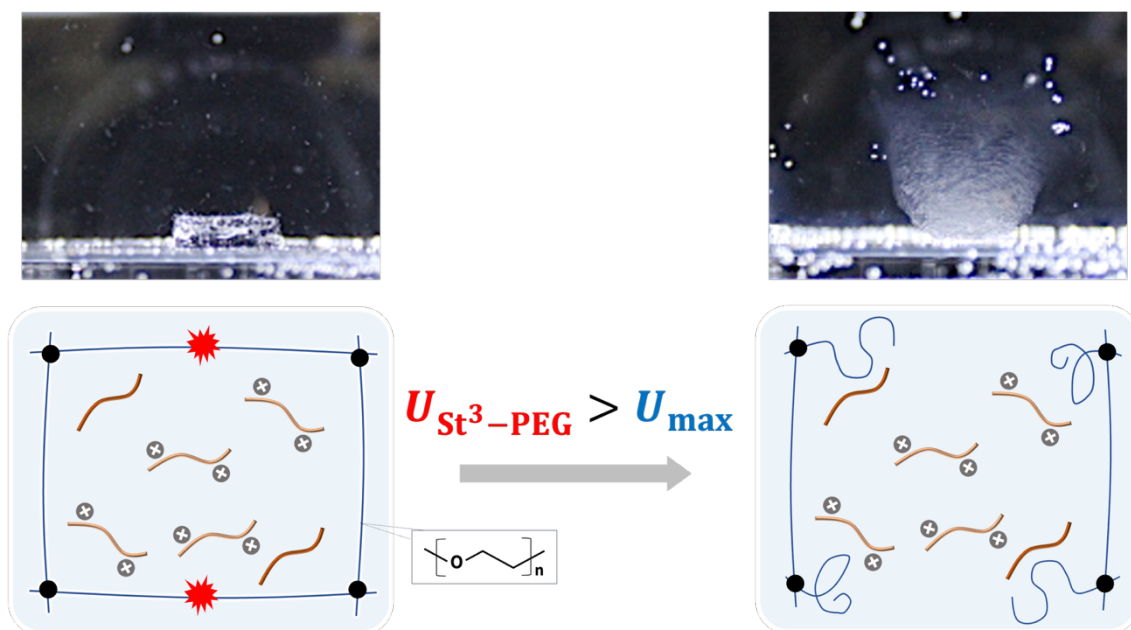


Figure 5-5. Illustration of osmotic explosion. Polymer scission occur when $U_{\text{st}^3\text{-PEG}}$ exceed U_{max} .

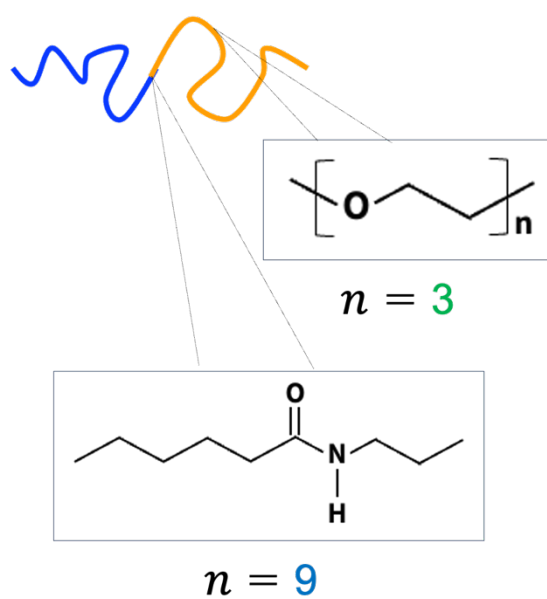


Figure 5-6. Number of bond per PEG strand in the Tetra-PEG gels.

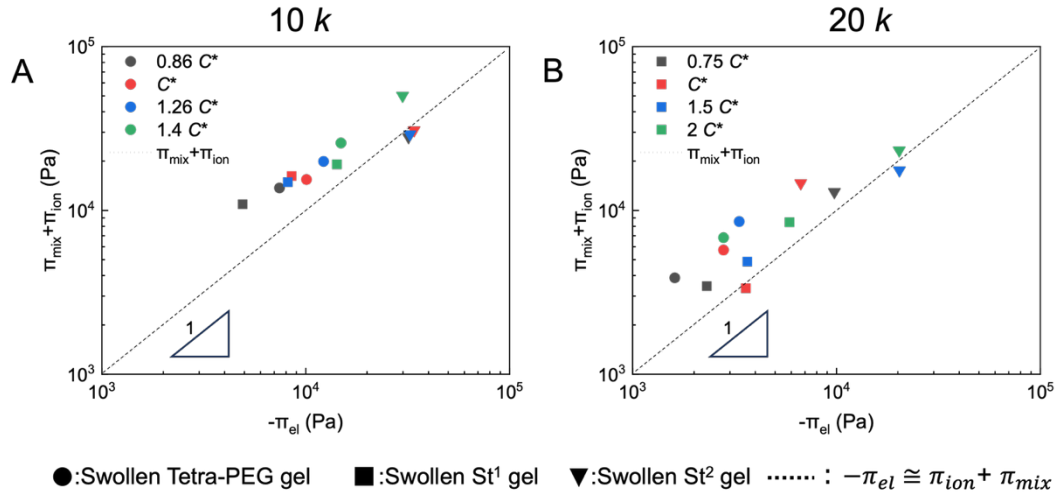


Figure 5-7. Relationship between the estimated osmotic pressure and elastic pressure. Osmotic pressure of the Tetra-PEG gels were estimated and plotted against their measured elastic pressure, π_{el} ; (A) 10 k, (B) 20 k. The dotted line shows the ideal osmotic pressure of a gel at swelling equilibrium. For the estimation of π_{mix} , the best fitting parameters for χ_{12} and χ_{13} are used to minimize the values of $(\pi_{mix} + \pi_{ion} + \pi_{el})^2$. The values are both 0. Figures Reproduced from *Small*, with permission from Wiley-VCH.

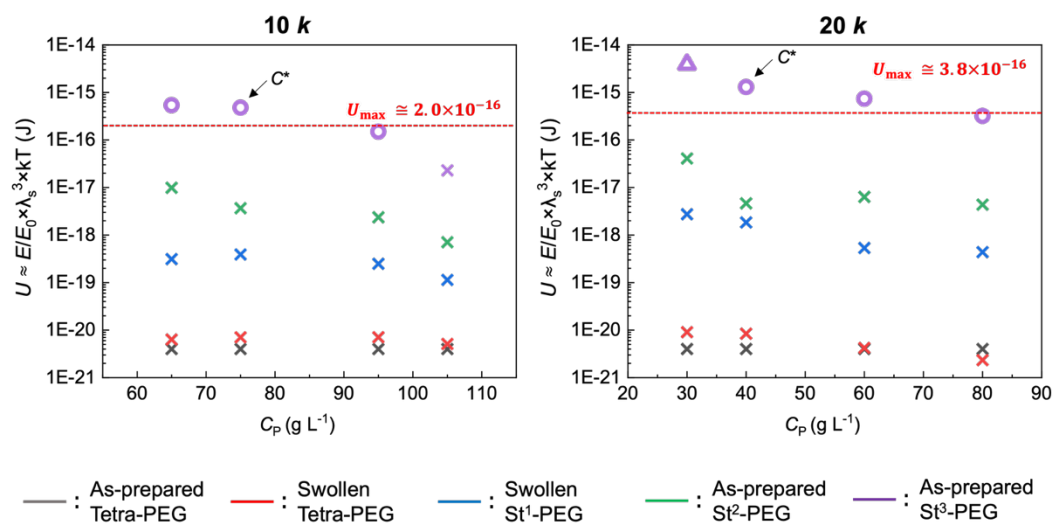


Figure 5-8. Estimated U and $U_{\max}$ and the occurrence of the disintegration of the St³-PEG gels. The circle and cross indicate fragmentation and no fragmentation, respectively. The triangle indicates that the St³-PEG gels were covered by oozed molecular stent but still maintained the gel state. C_p is prepolymer concentration, and C^* for each M_w is 75 (10 k), 40 (20 k) g L⁻¹, respectively. Figures Reproduced from *Small*, with permission from Wiley-VCH.

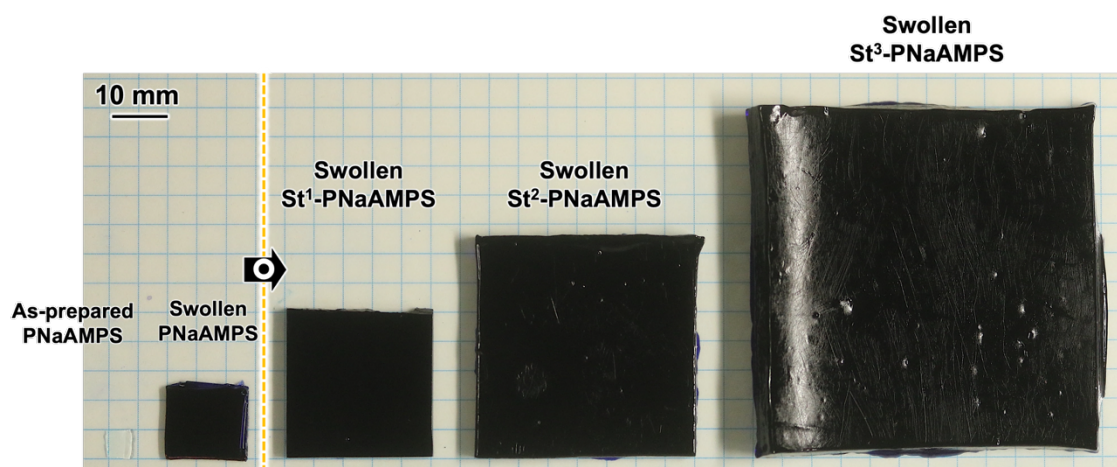


Figure 5-9. Pictures of the PNaAMPS gels and corresponding St gels. From the left is as-prepared PNaAMPS, swollen PNaAMPS, St¹, St², St³-PNaAMPS gels. The swollen gels were stained with methylene blue for visualization.

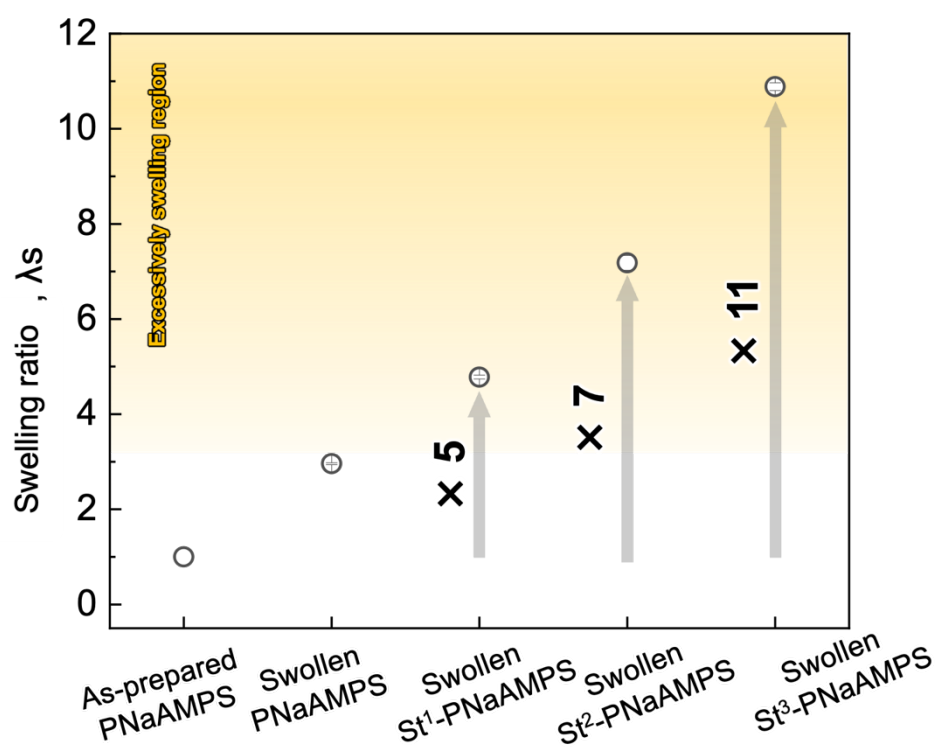


Figure 5-10. Uniaxial swelling ratio, λ_s , of the PNaAMPS gels and corresponding St¹, St², and St³-PNaAMPS gels.

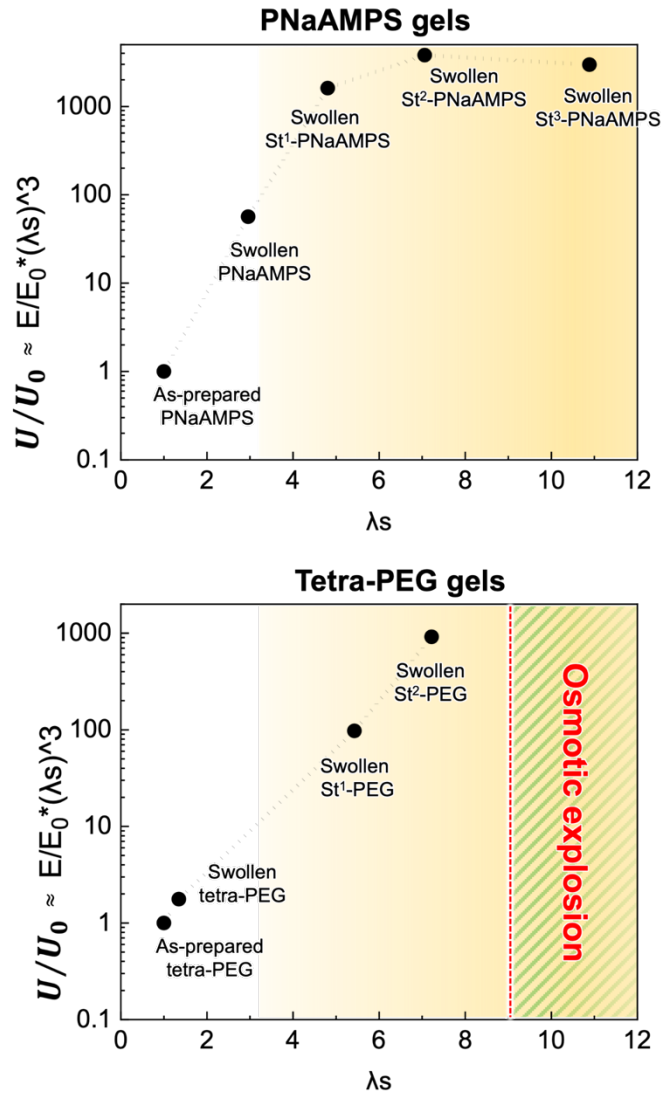


Figure 5-11. Normalized stored elastic energy per strand, U/U_0 , of the PNaAMPS and Tetra-PEG gels as a function of their uniaxial swelling ratio.

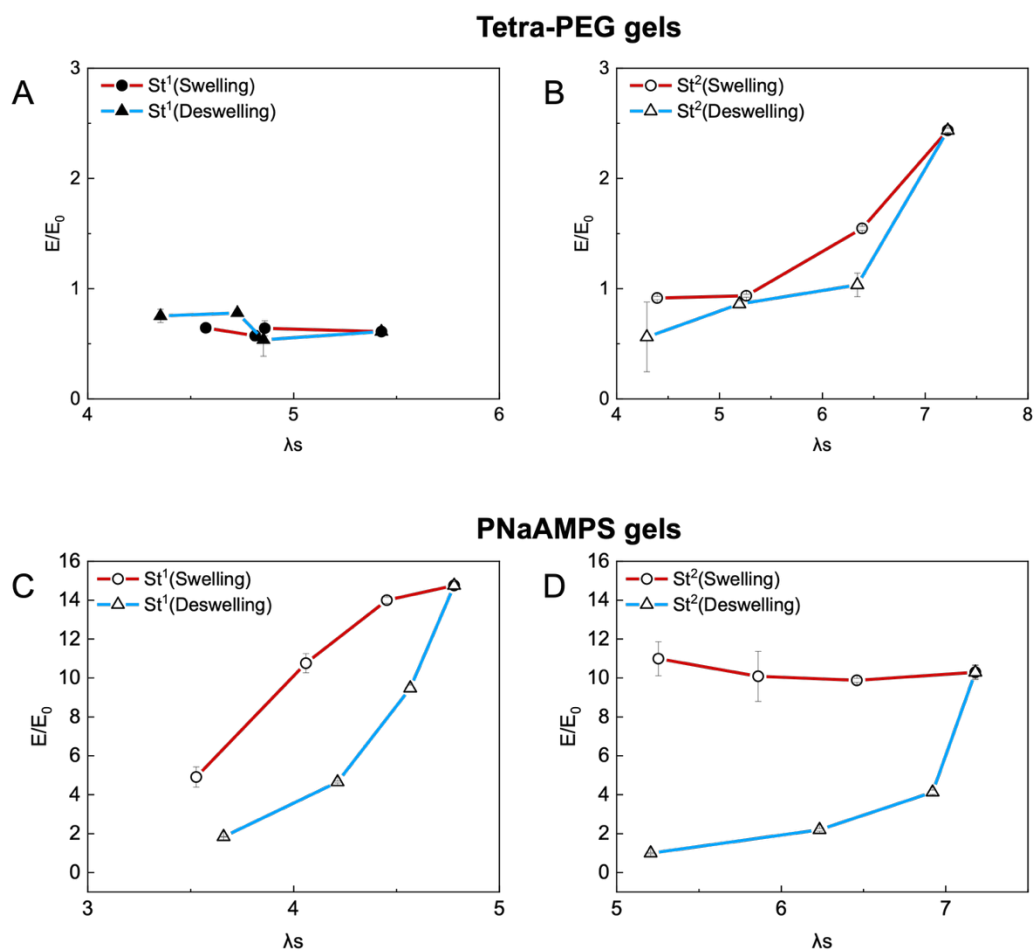


Figure 5-12. Normalized elastic modulus, E/E_0 , of the PNaAMPS and Tetra-PEG gels as a function of their uniaxial swelling ratio. The error bar represents mean $\pm$ SD ($N = 3$). Red line shows the swelling process and blue one shows the deswelling process.

5.5 References

1. Tominaga T., Tirumala V. R., Lin E. K., Gong J. P., Furukawa H. Osada Y. and Wu W.-L. “The molecular origin of enhanced toughness in double-network hydrogels: A neutron scattering study” *Polymer* **48**, 7449–7454 (2007).
2. Itagaki H., Kurokawa T., Furukawa H., Nakajima T., Katsumoto Y. and Gong J. P. “Water-Induced Brittle-Ductile Transition of Double Network Hydrogels” *Macromolecules* **43**, 9495–9500 (2010).
3. Nakajima T., Chida T., Mito K., Kurokawa T. and Gong J. P. “Double-network gels as polyelectrolyte gels with salt-insensitive swelling properties” *Soft Matter* **16**, 5487–5496 (2020).
4. Guo H., Kurokawa T., Takahata M., Hong W., Katsuyama Y., Luo F., Ahmed J., Nakajima T., Nonoyama T. and Gong J. P. “Quantitative Observation of Electric Potential Distribution of Brittle Polyelectrolyte Hydrogels Using Microelectrode Technique” *Macromolecules* **49**, 3100–3108 (2016).
5. Nishimura T., Guo H., Kiyama R., Katsuyama Y., Gong J. P. and Kurokawa T. “In Situ Evaluation of the Polymer Concentration Distribution of Microphase Separated Polyelectrolyte Hydrogels by the Microelectrode Technique” *Macromolecules* **54**, 10776–10785 (2021).
6. Lake G. J. and Thomas A. G. “The Strength of Highly Elastic Materials” *Proc. Roy. Soc. A* **300**, 108–119 (1967).
7. Kerr J. A. “Bond Dissociation Energies by Kinetics Methods” *Chem. Rev.* **66**, 465–500 (1966).
8. Marochkin I. I. and Dorofeeva O. V. “Amide bond dissociation enthalpies: Effect of substitution on N–C bond strength” *Comput. Theor. Chem.* **991**, 182–191 (2012).
9. Wang S., Panyukov S. V., Rubinstein M. and Craig S. L. “Quantitative Adjustment to the

Molecular Energy Parameter in the Lake-Thomas Theory of Polymer Fracture Energy” *Macromolecules* **52**, 2772–2777 (2019).

10. Treloar L. R. G. *The Physics of Rubber Elasticity* (Clarendon Press, Oxford, UK, 1976).
11. Nakajima T., Chida T., Mito K., Kurokawa T. and Gong J. P. “Double-network gels as polyelectrolyte gels with salt-insensitive swelling properties” *Soft Matter* **16**, 5487–5496 (2020).
12. Leslie K.-A., Robert D.-S., Arora S., Curley S. J., Szczepanski C. and Driscoll M. M. “Gel rupture during dynamic swelling” *Soft Matter* **17**, 1513–1520 (2021).
13. Akagi Y., Katashima T., Sakurai H., Chung U.-I. and Sakai T. “Ultimate elongation of polymer gels with controlled network structure” *RSC Advances* **3**, 13251–13258 (2013).
14. Ryuji Kiyama, Masahiro Yoshida, Takayuki Nonoyama, Tomáš Sedláčik, Hiroshi Jinnai, Takayuki Kurokawa, Tasuku Nakajima and Jian Ping Gong “Nanoscale TEM Imaging of Hydrogel Network Architecture” *Adv. Mater.* **35**, 2208902 (2023).

Chapter 6: Summary of this dissertation

6.1 General conclusions and outlook

We successfully achieved excess swelling of gels near or beyond their structural swelling limit through the multiple introductions of molecular stents. Near the structural swelling limit, the elastic modulus of the gels increases significantly due to the high elongation of the network strands, potentially enabling the development of low-density yet stiff polymeric materials. Beyond this limit, the over-swollen gels undergo osmotic explosion, fragmenting due to the rupture of network strands. Also, we demonstrated that the changes in physical properties upon swelling vary accordingly by using gels with different degrees of network strand uniformity. Furthermore, we found that the uniformity of network strands plays a crucial role in the occurrence of the *osmotic explosion*. This phenomenon could contribute to the development of polymeric materials that spontaneously degrade upon exposure to water after use.

This study highlights the remarkable ability of osmotic pressure to break down molecules and materials. Just as the high pressure generated by explosives can shatter buildings (macroscopic networks), high osmotic pressure can disintegrate microscopic macromolecular networks. This insight suggests the potential of osmotic pressure in applications such as osmotic milling for material processing, as well as in addressing environmental concerns through the osmotic decomposition of materials at the time of disposal.

Moreover, this work suggests the possibility of using swelling as a general tool to apply controlled mechanical energy to bulk polymeric materials.[1] Conventional methods for imparting mechanical energy to bulk materials, such as ultrasonication or ball milling, require significant energy input and offer limited control over the energy transferred. While the controlled extension of individual polymer chains has been achieved using atomic force

microscopy,[2–4] and local swelling or surface tension can induce the stretching of (hyper)branched polymers,[5–7] these approaches are limited to single-molecule systems and are not applicable to bulk materials. In contrast, our multiple molecular stent method can, in principle, deform macroscopic gel networks composed of various chemical species to any desired extent by regulating the swelling. The mechanical energy accumulated in the network strands can be tuned via the degree of swelling and predicted from the bulk elastic modulus. Importantly, no additional external energy input is required during the fragmentation process in solution—once the molecular stents have been introduced—although UV irradiation is necessary for their initial incorporation. This method simply requires immersion of the gel into a solution and does not rely on external energy sources during swelling. We anticipate that this approach will significantly advance the field of swelling-induced mechanochemistry, particularly through the implementation of our multiple molecular stent strategy.

6.2 References

1. Metze F. K., Sant S., Meng Z., Klok H.-A. and Kaur K. “Swelling-Activated, Soft Mechanochemistry in Polymer Materials” *Langmuir* **39**, 3546–3557 (2023).
2. Rief M., Oesterhelt F., Heymann B. and Gaub E. “Single Molecule Force Spectroscopy on Polysaccharides by Atomic Force Microscopy” *Science* **275**, 1295–1297(1997).
3. Oesterhelt F., Rief M. and Gaub H. E. “Single molecule force spectroscopy by AFM indicates helical structure of poly(ethylene-glycol) in water” *New J. Phys.* **1**, 6.1–1.11 (1999).
4. Aktah D. and Frank I. “Breaking Bonds by Mechanical Stress: When Do Electrons Decide for the Other Side?” *J. Am. Chem. Soc.* **124**, 3402–3406 (2002).
5. Li Y., Nese A., Lebedeva N. V., Davis T., Matyjaszewski K. and Sheiko S. S. “Molecular Tensile Machines: Intrinsic Acceleration of Disulfide Reduction by Dithiothreitol” *J. Am. Chem. Soc.* **133**, 17479–17484 (2011).
6. Messmer D., Bertran O., Kissner R., Alemán C. and Schlüter D. “Main-chain scission of individual macromolecules induced by solvent swelling” *Chem. Sci.* **10**, 6125–6139 (2019).
7. Wang J. and Klok H.-A. “Swelling-Induced Chain Stretching Enhances Hydrolytic Degrafting of Hydrophobic Polymer Brushes in Organic Media” *Angew. Chem. Int. Ed.* **58**, 9989–9993 (2019).

List of Publications

Original papers

1. Shou Ohmura, Tasuku Nakajima, Masahiro Yoshida, and Jian Ping Gong "Squid/synthetic polymer double-network gel: elaborated anisotropy and outstanding fracture toughness characteristics", *NPG Asia Materials*, 2023, 15, 2
2. Shou Ohmura, Kosei Oikawa, Takuya Nishimura, Ryuji Kiyama, Jian Ping Gong, Tasuku Nakajima: "Osmosis-Induced Mechanical Disintegration of Polymer Network Gels", *Small*, 2025, 2503209

Other papers

1. Yuxin Cheng, Stephen Lee, Yihang Xiao, Shou Ohmura, Louis-jacques Bourdages, Justin Puma, Zixin He, Zhen Yang, Jeremy Brown, Jean Provost, Jianyu Li "Ultrasound Cavitation Enables Rapid, Initiator-Free Fabrication of Tough Anti-Freezing Hydrogels" *Adv. Sci.* 2025, 2416844

Press coverage

1. 大村将、中島祐、吉田匡宏、グン剣萍：「イカと合成高分子の複合により耐破壊性ハイドロゲルを開発！！」 *Chem-station*, 2023
2. 北大、「イカ」と人工ポリマーをハイブリッド化 高強度素材を開発 *Nikkan Kogyo Shimbun*
3. 生物に学ぶ イカの切り身で医療材料 *Nippon Keizai Shimbun*

Presentation in conferences

1. 大村将、中島祐、グン剣萍：「天然物のメソスケール構造を生かした高機能・高靱性ゲルの創製」高分子学会、第56回高分子学会北海道支部研究発表会（令和4年1月24日、オンライン、O04）

2. 大村将、中島祐、グン剣萍：「天然物のメソスケール構造を生かした高機能・高靱性ゲルの創製」高分子学会、第71回高分子学会年次大会（令和4年5月26日、オンライン、P035）
3. Shou Ohmura、Tasuku Nakajima、Jian Ping Gong “Squid/Polyacrylamide anisotropic DN gel with high fracture toughness” 高分子学会、13th International Gel Symposium（令和4年9月3日、北海道、P6）
4. 大村将、中島祐、グン剣萍：「超膨潤によるゲルの分解」高分子学会、第72回高分子学会年次大会（令和5年5月24日、群馬、1Pc049）
5. Shou Ohmura、Tasuku Nakajima、Jian Ping Gong “Super swelling induces degradation of hydrogels” 高分子学会、13th International Polymer Conference（令和5年7月19日、札幌、19P061a）
6. 大村将、吉田匡宏、中島祐、グン剣萍：「高分子鎖の極限伸長に伴うゲルの硬化と分解の理論的解析」高分子学会、第72回高分子討論会（令和5年9月27日、香川、2H06）

Poster prize

1. Soft Matter Prize Award (13th International Gel Symposium)

Patent

- 1 Patent related to the doctoral thesis

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