Hydrogels

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Non-Osmotic Hydrogels: A Rational Strategy for Safely Degradable Hydrogels

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Abstract: *Hydrogels are promising materials for biomedical applications, where timely degradation is often preferred. In the conventional design, however, the cleavage of polymer networks essentially causes considerable morphological changes (i.e., degradation-induced swelling), triggering various medical complications. Herein, we report a rational strategy to suppress the degradation-induced swelling based on the synthetic control of the polymer–solvent interaction parameter (χ) of constituent polymer networks. The resultant hydrogels with an optimal χ parameter ($\chi_{37°C} \approx 0.53$; non-osmostic hydrogels) displayed the capability to retain their original shape and degrade without generating significant swelling pressure under physiological conditions ($\Pi_{37°C} < 1$ kPa). This concept of the safely degradable non-osmotic hydrogel is theoretically universal, and can be exploited for other types of synthetic hydrogels in various settings.*

Hydrogels, referring to cross-linked polymer networks swollen in water, are considered a promising tool to build implantable biomaterials, such as temporary scaffolds for cells or drug reservoirs in the field of regenerative medicine.[1–3] After completing all the programed tasks (e.g., complete release of loaded drugs), hydrogels are required to degrade, and to be cleared out of the body. Although the recent progress in the development of degradable hydrogels is remarkable,[4–6] intended or unintended cleavages of polymer network strands inevitably results in macroscopic changes in shape (i.e., degradation-induced swelling).[7] This is even the case for the emerging swelling-controlled hydrogels, in which the initial swelling is suppressed via special mechanisms.[8–10] Serious risks arising from the hydrogel swelling include undesirable nerve compression, damage to neighboring tissues, and displacement from the installation site. Therefore, it seems reckless to apply conventional degradable hydrogels to semi-confined in vivo spaces.

The theoretical aspects of the swelling behavior must be carefully contemplated in order to realize the unconditional suppression of swelling (i.e., independent of the degree of degradation). The Flory–Rehner theory is one of the most powerful tools to predict the equilibrium swelling state, in which the pressure applied to hydrogels becomes zero.[11] Since the pressure is simply given by the sum of the osmotic (Π_{os}) and elastic (Π_{el}) pressures for neutral hydrogels, the equilibrium can be written as Equation (1).

$$\prod_{os} + \prod_{el} = 0 \tag{1}$$

For hydrogels that contain elastically effective chains at the concentration of ν (mol m^{-3}), these pressures can be written as shown in Equations (2) and (3),

$$\prod_{os} = -\frac{kT}{V_s}\left(\phi + \ln(1-\phi) + \chi\phi^2\right) \tag{2}$$

$$\prod_{el} = \nu kT\left(\frac{1}{2}\frac{\phi}{\phi_0} - \left(\frac{\phi}{\phi_0}\right)^{\frac{1}{3}}\right) \tag{3}$$

where ϕ_0 is the initial polymer volume fraction, ϕ is the equilibrium polymer volume fraction, χ is the polymer-solvent interaction parameter, and V_s is the molar volume of the solvent. The degree of swelling (Q) is given by ϕ_0/ϕ. By substituting Equation (2) and Equation (3) into Equation (1), we obtain the following relationship given in Equation (4).

$$\nu = \frac{-\left(\ln\left(1-\frac{\phi_0}{Q}\right) + \frac{\phi_0}{Q} + \chi\left(\frac{\phi_0}{Q}\right)^2\right)}{V_s\left(-\frac{1}{2}Q^{-1} + Q^{-\frac{1}{3}}\right)} \tag{4}$$

According to the affine network model, ν is related to the elastic modulus (E) as $E/3 = \nu RT$ (R: gas constant).[12] Using Equation (4), we can draw the relationship between elastic modulus and the degree of swelling as a function of χ (Figure 1a). The theory states that hydrogels with $\chi < 0.53$

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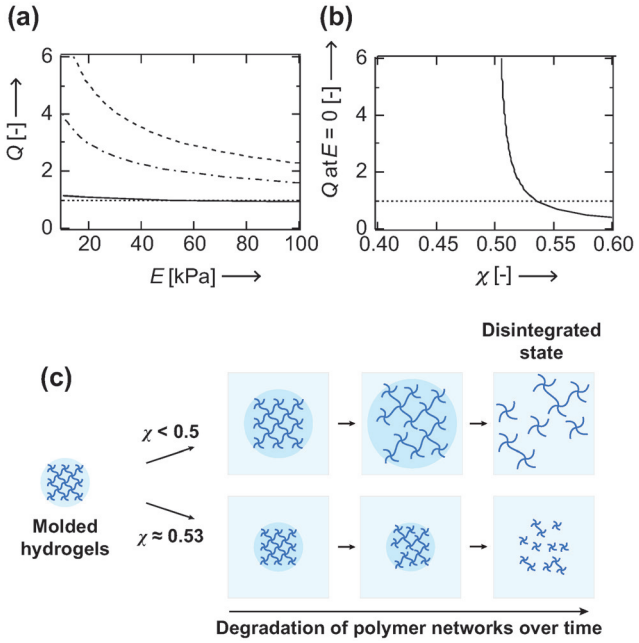


Figure 1. Theoretical prediction of the degradation behavior. a) Q as a function of E calculated for hydrogels with $\phi_0 = 0.10$. Each line represents $\chi = 0.45$ (dashed line), 0.49 (dash-dot line) and 0.53 (solid line). The dotted line represents the state of non-swelling, $Q = 1$. b) Q of hydrogels with $\phi_0 = 0.10$ at the disintegration point ($E = 0$) as a function of χ. c) Conceptual illustration of different degradation routes depending on χ.

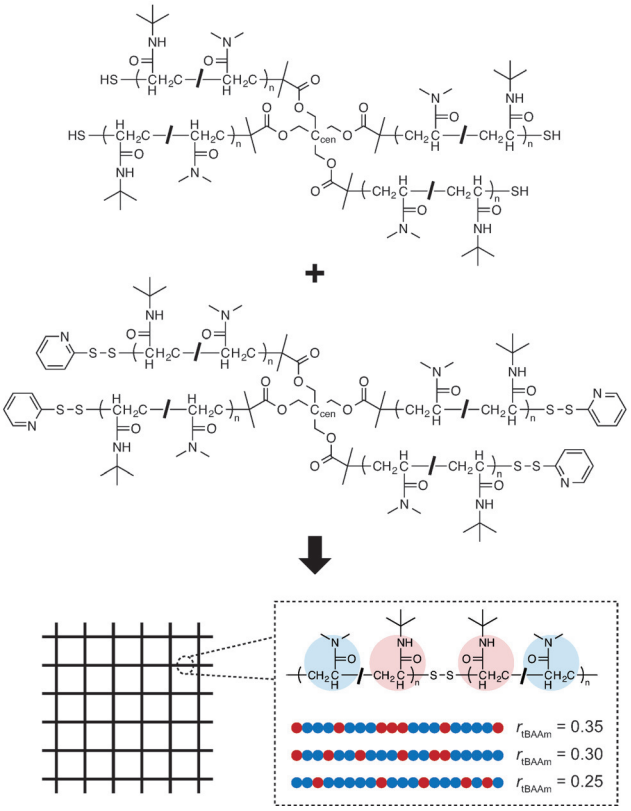
swell ($Q > 1$) in the region of elastic modulus typical for biological soft tissues ($E < 100$ kPa).[13] Notably, even in the region $0.50 < \chi < 0.53$, which is more hydrophobic than the θ state ($\chi = 0.5$), hydrogels exhibit swelling tendency; therefore, the θ state is not the boundary between swollen and shrunken state. The swelling tendency is more pronounced as the elastic modulus decreases (Figure 1a, dashed and dash-dot lines), which qualitatively corresponds to the degradation process of polymer networks. In stark contrast, the swelling of hydrogels with higher χ parameters (e.g., $\chi = 0.53$) is minimized ($Q \approx 1$, irrespective of E) (Figure 1a, solid line), which is referred to as the non-osmotic state—where $\Pi_{os} = 0$. Furthermore, we investigated the swelling state of hydrogels at the disintegration point by substituting 0 into the elastic term ν in Equation (4) [Eq. (5)].

$$\chi = \frac{-\left(\ln\left(1-\frac{\phi_0}{Q}\right)+\frac{\phi_0}{Q}\right)}{\left(\frac{\phi_0}{Q}\right)^2} \tag{5}$$

We calculated the analytical solution of Equation (5) for hydrogels with $\phi_0 = 0.10$, which represent hydrogels that contain approximately 90% water. Figure 1b indicates that hydrogels with $\chi < 0.5$ swell to infinite degrees at the moment of disintegration, while on the other hand, those with $\chi > 0.5$ show finite degrees of swelling even when completely disintegrated. This analysis also suggests that an optimal value of χ for maintaining $Q = 1$ is somewhere in the vicinity of 0.53, which reveals that the polymer species employed in conventional hydrogels are too hydrophilic to be used as building modules for hydrogels that need to maintain a constant degree of swelling. For example, χ parameters of poly(ethylene glycol) and poly(acrylamide) are 0.45 and 0.49, respectively.[14,15] In summation, building hydrogels from polymers with $\chi \approx 0.53$ is theoretically required to achieve constant swelling properties for degrading hydrogels (Figure 1c).

Here, we report a rational strategy for achieving hydrogels that are safely degradable with non-burst swelling pressure. Our practical approach to realize the non-osmotic state under physiological conditions was based on the synthetic control of χ by synthesizing copolymers from monomers with different hydrophobicity: *N*,*N*-dimethylacrylamide (DMAAm) as a hydrophilic monomer, and *N*-*tert*-butylacrylamide (tBAAm) as a hydrophobic monomer, and the subsequent build up of hydrogels from the polymer units. In this study, we synthesized two types of four-armed polymers that are mutually reactive to their counterparts via the thiol-disulfide exchange. The simple mixing of aqueous solutions of prepolymer units instantaneously produced hydrogels with controlled hydrophobicity (tetra-poly(DMAAm-*co*-tBAAm) hydrogels) (Scheme 1).

The swelling behavior of tetra-poly(DMAAm-*co*-tBAAm) hydrogels varied depending on r_{tBAAm}. Q of hydrogels with different r_{tBAAm} values at each temperature is shown



Scheme 1. Chemical structures of polymer units and cross-linked polymer networks. Hydrophilic and hydrophobic monomers are depicted as blue and red spheres, respectively. The hydrophobic monomer ratio is expressed as r_{tBAAm}. For experimental details, see the Supporting Information.

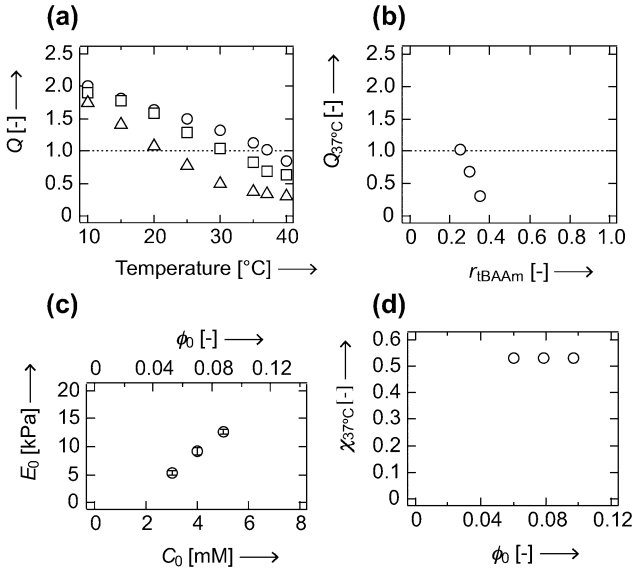
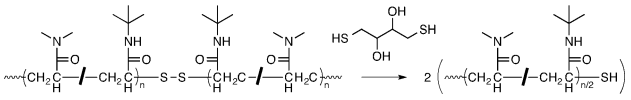


Figure 2. Physical properties of tetra-poly(DMAAm-*co*-tBAAm) hydrogels. a) Q as a function of temperature. ϕ_0 was fixed at 0.0965. The symbols represent $r_{tBAAm} = 0.25$ (circle), 0.30 (square) and 0.35 (triangle). b) Q at 37 °C ($Q_{37°C}$) as a function of r_{tBAAm}. c) Elastic modulus of as-prepared hydrogels (E_0) with $r_{tBAAm} = 0.25$ as a function of the initial concentration of prepolymer units (C_0) or ϕ_0. d) χ at 37 °C ($\chi_{37°C}$) of hydrogels with $r_{tBAAm} = 0.25$ as a function of ϕ_0.



Scheme 2. Reductive cleavage of the polymer network strands of non-osmotic hydrogels by dithiothreitol.

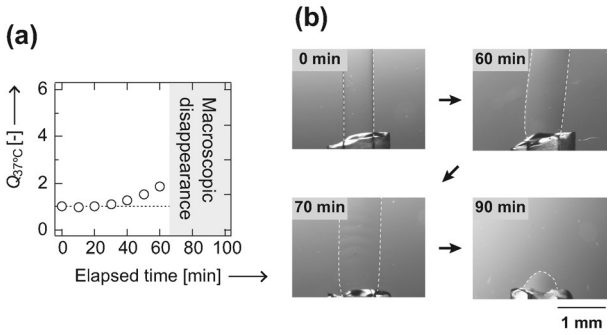


Figure 3. Degradation behavior of non-osmotic hydrogels. a) Time course change in Q of a hydrogel with parameters $r_{tBAAm} = 0.25$ and $\phi_0 = 0.0965$ at 37 °C after the addition of dithiothreitol. b) Representative photos of degrading hydrogels (outlined with dashed lines) taken at specified time points.

in Figure 2a. At the constant temperature of 37 °C, the degree of swelling was a decreasing function of r_{tBAAm}, demonstrating the non-swelling property ($Q \approx 1$) at $r_{tBAAm} = 0.25$ (Figure 2b). It should be noted that, pure poly(DMAAm) gels (hydrogels with $r_{tBAAm} = 0$) are known to swell ($Q \gg 1$) over this whole temperature range due to their hydrophilic nature.[16] χ can be obtained, according to Equation (4), if we know ν in addition to the information about the swelling behavior discussed earlier. We prepared hydrogels with different ϕ_0 values, employing polymer units with $r_{tBAAm} = 0.25$, and conducted elongation tests. E increased proportionally with ϕ_0 (Figure 2c). This reflects the crosslinking nature of this hydrogel formation system, in which each polymer unit works as a crosslinking point; the increment of ϕ_0 leads to the increase in ν, consequently increasing E. The χ of hydrogels with $r_{tBAAm} = 0.25$ at 37 °C ($\chi_{37°C}$) was almost constant at approximately 0.53 (Figure 2d), which precisely corresponds to the theoretical prediction discussed above. It was, therefore, experimentally demonstrated that hydrogels with $\chi \approx 0.53$ exhibit unconditionally suppressed swelling properties (i.e., non-osmotic hydrogels).

The degradation behavior of non-osmotic hydrogels is completely different from that of the conventional hydrogels—i.e., those made of so-called hydrophilic polymers. To characterize the effect of the loss of elastic energy (i.e., decrement in ν) on the swelling behavior, we intentionally cleaved the crosslinking disulfides by the addition of dithiothreitol, a reducing agent (Scheme 2), and examined the consequent swelling behavior. The time course of the change in Q after the addition of reducing agents was recorded (Figure 3a, left axis), which showed that the volume of non-osmotic hydrogels was constant at the early stage. The boundary gradually became obscure over time, while the volume slightly increased. To characterize the effect of this slight swelling, the swelling pressure ($\Pi_{37°C}$) of the degrading hydrogels ($Q \approx 2$) was analyzed by immersing hydrogels in an aqueous solution that contained poly(vinylpyrrolidone), the osmotic pressure of which is already known,[17] which resulted in $\Pi_{37°C} = 0.58$ kPa. Considering the fact that the elastic modulus of biological soft tissues is at least in the order of a few kilopascals,[18,19] one can safely say that the degradation-induced swelling of non-osmotic hydrogels would be effectively suppressed in semi-confined in vivo spaces. After the slight swelling took place, we directly observed the macroscopic disappearance of non-osmotic hydrogels without detecting further obvious swelling (Figure 3b). This degradation behavior is noticeably different from that of conventional hydrogels, which significantly increase their volumes before reaching their disintegration points—typically, $Q > 8$.[5]

Introducing functional moieties to polymer networks can further extend the functionality of non-osmotic hydrogels. We introduced RGD peptides, which are cell adhesive ligands, to the polymer network in order to demonstrate the versatility and the cytocompatibility of non-osmotic hydrogels (RGD-conjugated non-osmotic hydrogels) (Figure 4a). MCF-10A human mammary epithelial cells, used extensively as a model cell line for various tissue engineering applications,[20] were seeded on the gel surface. After two days of culture, clear cell adhesion and spreading were observed from the cell morphology, while little to no cell adhesion was observed on the unmodified non-osmotic hydrogels, demonstrating the specific induction of cell adhesion through the RGD functionalization (Figure 4b, top). The cytocompatibility of non-

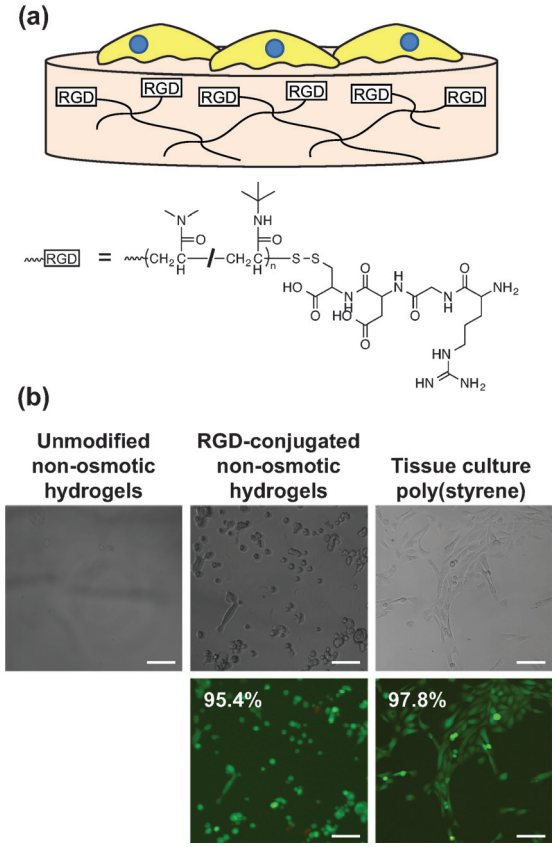


Figure 4. Cell viability tests on non-osmotic hydrogels. a) Schematic illustration of cell adhesion on RGD-conjugated non-osmotic hydrogels. b) Bright-field images (top) and live/dead assay (green = alive, red = dead) (bottom) of MCF-10A cells cultured for two days on unmodified non-osmotic hydrogels (left), RGD-conjugated non-osmotic hydrogels (center), and tissue culture poly(styrene) dish as a control (right). Percentages indicate the viability of cells (number of live cells/number of total cells × 100%). Scale bars indicate 100 μm.

osmotic hydrogels were also confirmed by live/dead assays of the adhesion-dependent MCF10A cells cultured on the RGD-conjugated non-osmotic hydrogels, which demonstrated excellent cell viability (95.4%) (Figure 4b, bottom). As such, non-osmotic hydrogels have immense potentials to become cell-friendly materials for various biomedical applications.

In summary, a new material design for safely degradable hydrogels was proposed, and the validity of the proposed strategy was experimentally confirmed via the synthesis of hydrogels with an optimal χ (non-osmotic hydrogels). Non-osmotic hydrogels have, at least, three advances over conventional non-swellable hydrogels,[9] which contain thermoresponsive polymer units. One is the unconditional suppression of swelling. As long as polymers with $\chi = 0.53$ are used as a constituent polymer, resultant hydrogels always show non-swelling behavior under physiological conditions, regardless of the network structure. Importantly, degradation-induced swelling is also inhibited. Second, strict temperature control in the preparation stage is not required in this design; a relatively low temperature condition is required for preparing previous non-swellable hydrogels. It will greatly help the formation of hydrogels directly in vivo. The third is the absence of precipitation due to disintegrated polymer species. In contrast to the thermoresponsive polymers used in non-swellable hydrogels, the constituent polymers of non-osmotic hydrogels can dissolve into physiological solutions, which overcomes the drawbacks caused by the precipitation of polymer chains. We envision that the concept of non-osmotic hydrogels can be extended to any types of synthetic hydrogels, and could significantly benefit the future development of safely implantable drug carriers, surgical sealants and in vivo three-dimensional culture systems, where programmed degradation without affecting surrounding tissues is required.

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