Highly Stretchable and Notch-Insensitive Hydrogel Based on Polyacrylamide and Milk Protein

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ABSTRACT: Protein-based hydrogels have received attention for biomedical applications and tissue engineering because they are biocompatible and abundant. However, the poor mechanical properties of these hydrogels remain a hurdle for practical use. We have developed a highly stretchable and notch-insensitive hydrogel by integrating casein micelles into polyacrylamide (PAAm) networks. In the casein-PAAm hybrid gels, casein micelles and polyacrylamide chains synergistically enhance the mechanical properties. Casein-PAAm hybrid gels are highly stretchable, stretching to more than 35 times their initial length under uniaxial tension. The hybrid gels are notch-insensitive and tough with a fracture energy of approximately 3000 J/m². A new mechanism of energy dissipation that includes friction between casein micelles and plastic deformation of casein micelles was suggested.

KEYWORDS: tough hydrogels, polyacrylamide, casein, protein hydrogels, notch-insensitive hydrogels, energy dissipations

Protein-based hydrogels are attractive candidates for biomedical applications¹,² and tissue engineering³−⁵ because of their unique properties such as biocompatibility, responsiveness to various kinds of stimuli, and abundance. However, hydrogels based on protein generally show low stretchability and brittleness with low toughness.⁶−⁹ A few strategies have been attempted to enhance the mechanical properties of the protein-based hydrogels, such as force-unfolded protein hydrogels,¹⁰ human tropoelastin-graphene oxide composite hydrogels,¹¹ and silk microsphere-reinforced silk hydrogel.¹² However, the mechanical properties of these gels are still insufficient for applications requiring large deformations and high toughness.

To toughen a hydrogel, researchers have suggested various mechanisms over the last few decades, such as double network hydrogels,¹³ nanocomposite hydrogels,¹⁴ macromolecular microsphere composite hydrogels,¹⁵ and polyampholyte hydrogels.¹⁶ The double-network hydrogels are composed of primary networks (highly cross-linked) and secondary networks (loosely cross-linked). The secondary networks contribute shape retention, and the primary networks dissipate the energy applied to the gels.¹⁷,¹⁸ The nanocomposite hydrogels exhibited high stretchability due to the high functionality of the exfoliated nanoclay. Multiple polymer chains are connected to two adjacent high-functionality cross-linkers, and these chains usually have nonuniform lengths. Relatively long chains maintain the elasticity of the nanocomposite hydrogels and the short chains participate in energy dissipation. Alternately, a macromolecular microsphere can be used as both an initiator and a cross-linker, similar to the nanocomposite. Polyampholyte hydrogels have both strong ionic bonds and weak ionic bonds between long ampholyte polymer chains. In this structure, the strong ionic bonds maintain the shape of the gels, and the weak ionic bonds dissipate energy by debonding. Therefore, the key factors to toughen a hydrogel are shape retention and energy dissipation.¹⁹−²²

Casein is a major protein component of bovine milk, consisting of αs1-, αs2-, β-, and κ-caseins in approximate relative amounts of 4:1:3.5:1.5, respectively. Casein possesses several beneficial characteristics suitable for biomaterials, such as good biodegradability and availability of reactive sites for chemical modification.²³,²⁴ Casein forms a micellar structure in a hydrated state. Most of the κ-casein, which is a hydrophobic compound, is found on the surfaces of the casein micelles.²⁵ With acidification, which decreases the negative charge−charge repulsion on the casein micelle, casein forms a 3-dimensional (3D) coagulated structure by a hydrophobic association. A casein micelle consists of a number of submicelles which can be rearranged by applying a shear force. By this rearrangement of submicelles, a casein micelle can possibly deform plastically and as a result might have the capability to dissipate energy. Casein micelles would be good candidates for sacrificial architecture in tough hydrogels. Previously, hydrogels composed of PAAm and casein protein have been reported for the purpose of drug release.²⁶ However, these hydrogels only possessed a PAAm...
network without the 3D structure of casein. Hence, mechanical energy dissipation was not possible.

In this study, we have presented a simple method for the synthesis of tough casein-polyacrylamide hydrogels. The casein-PAAm hydrogel was prepared by an acidification of casein micelles and a radical polymerization of acrylamide (AAm). We introduced a new strategy for toughening a gel: deformation energy was dissipated by friction between the micelles and by plastic deformation of the micelles. The process of synthesis was described, and the mechanical properties of the synthesized hydrogels were explored.

■ EXPERIMENTAL SECTION

Casein-PAAm hydrogels were prepared by a free radical polymerization of AAm and a coagulation of casein micelles, as shown in Figure S1. An aqueous solution of AAm with N,N'-methylenebis(acrylamide) (MBAA) was prepared. A casein solution was prepared by dissolving casein powder in deionized (DI) water. The pH of the casein solution was adjusted to 6.6 by the addition of NaOH solution. After the two stock solutions were mixed, glucono delta-lactone (GDL) powder was added as an acidifier to gradually decrease the pH. Ammonium persulfate (APS) as the radical initiator and N,N,N',N'-tetramethylenediamine (TEMED) as the cross-linking accelerator for AAm were added to the solutions. Subsequently, the solutions were poured into a glass mold. The solutions were then cured with 8 W, 254 nm wavelength UV light for 2 h and left for 24 h for casein gelation before mechanical testing.

Other details regarding the materials, the detail synthetic process, the mechanical tests, the scanning electron microscope (SEM) observation, the swelling experiments in DI water and phosphate-buffered saline (PBS), and the transmission electron microscope (TEM) observation are supplied in the Supporting Information.

■ RESULT AND DISCUSSION

We have synthesized stretchable and notch-insensitive hydrogels by integrating two types of polymers. Coagulation of the casein micelle is followed by a radical polymerization to form polyacrylamide, as shown in Figure S1. Casein shows a unique micellar structure in a hydrated state. \(\kappa\)-casein, which has a negatively charged hairy layer under neutral pH, is located on the surface of the casein micelles, such that a repulsive force between casein micelles contributes to the steric stabilization. With acidification, the net negative charge decreases, decreasing both the electrostatic repulsion and the steric stabilization. Therefore, the charged hairs shrink and casein micelles coagulate through a local hydrophobic association. Due to the hydrophobicity of the casein micelle, degree of the swelling of the hybrid gels decreased as the amount of the casein increased in the casein-PAAm hydrogels (Figure S2). It should be noted that the PAAm chains were formed by radical polymerization before coagulation of the casein micelles to allow synthesis of the casein-PAAm hydrogel without global phase separation.

As shown in Figure 1, the casein-PAAm hydrogels were highly stretchable and sufficiently tough to be used for complex deformations. The deformation behaviors under various stress conditions were very stable even though the initial thickness of
the samples was only 3 mm. It can be seen that knotted casein-PAAm hydrogels elongated to more than 13 times of their original length without fracture (Figure 1a, b). Furthermore, the areal strain of the casein-PAAm hydrogel exceeded 2500% (Figure 1c, d). The casein-PAAm hydrogel was also suitable for complex deformations. A gel with a diameter of only 90 mm was used to cover a cylinder with a diameter of 70 mm and a height of 210 mm (Figure 1e). Those experiments demonstrated that the casein-PAAm hydrogel was stable under the various deformation conditions without any distinct failure. The casein-PAAm hydrogels are extremely stretchable and can be applied to various stress states.

We performed a tensile test to observe the mechanical properties of the hydrogels (Figure 1f inset). Casein-PAAm hydrogel could be stretched to over 30 times of its original length without any visible rupture (Figure 1f). We studied the casein-PAAm hydrogels with different casein to casein plus acrylamide weight ratios (Figure 1f, g). $\alpha$ is the weight percentage of casein to casein plus acrylamide. The stretch at rupture increased dramatically in the case of 32 wt % of $\alpha$. A rigid hydrogel cannot be formed for $\alpha > 40$ wt % due to the low AAm contents. There was little effect of variation of composition on the elastic modulus of the casein-PAAm hydrogels at small strain. Because of the very low concentration of cross-linker, the casein-PAAm hydrogels show low elastic modulus (<12 kPa). To investigate the effects of chemical cross-linking of PAAm, we measured mechanical properties for the casein-PAAm hydrogel with various MBAA concentrations (Figure S3a, b). As the concentration of MBAA increased, the stiffness of the casein-PAAm hydrogel increased. However, the stretchability of the casein-PAAm hydrogel decreased dramatically with increasing concentration of MBAA, showing the highest stretchability with the minimum MBAA concentration. The hydrogels demonstrate a trade-off between the elastic modulus and stretchability. In addition, notch-insensitivity of casein-PAAm hydrogels tended to decline as the concentration of MBAA increased. Mechanical properties were highly affected by the pH of the casein-PAAm hydrogels (Figure S4). Rupture stretch and strength of the casein-PAAm hydrogels decreased as the pH of the casein stock increased. This occurs because casein micelle cannot build a strong network at high pH condition. The low pH required for favorable synthesis of the hybrid gel would be a hurdle for applications under physiological conditions. In Figure S5, we compared the casein-PAAm hydrogel with other recently reported tough hydrogels (Figure 2a–f). Both pure acrylamide hydrogel and pure casein hydrogel were totally broken and crushed after the 90% strain compressive test (Figure 2a–f). However, there was no visible damage to the casein-PAAm hydrogel at 90% compression (Figure 2g–i). When compressive loading is applied to a gel, cracks propagate by the highly concentrated...
stress at small internal or external cracks. Single network hydrogels, such as the pure PAAm and the pure casein hydrogels in this study, are susceptible to crack propagation because there is no mechanism to relieve the concentrated stress in those materials. That is, the small cracks in those hydrogels can easily turn into running cracks. In contrast, a crack in the casein-PAAm hydrogel cannot turn into a running crack but it is blunted immediately. When stress is applied to the gels, hydrophobic associations between the casein micelles start to break, and the casein micelles might deform plastically by shear force. This energy dissipation makes the crack edgeless and the hydrogels tough.

We stretched the casein-PAAm hydrogel with 5 notches (3 internal notches, 2 external notches, 5 mm length each) to demonstrate the notch-insensitivity of casein-PAAm hydrogel (Figure 3a). The notched casein-PAAm hydrogel was stretched to over 28 times of its initial length. When casein-PAAm hydrogel was stretched, the notch tips were blunted, and they remained in a stable state. Additionally, we prepared the casein-PAAm hydrogels with notch sizes ranging from 15 to 60 mm (Figure 3b) and measured the extension properties of those hydrogels. The tensile load–extension curves were adopted instead of the conventional stress–stretch curve. Because the deformations of notched samples were not homogeneous, the stress field of the sample was nonlinear throughout the entire area. It was surprising that the rupture stretch of casein-PAAm hydrogel is almost constant, insensitive to the notch length.

As notch-insensitivity results from the high fracture energy and large plastic zone near the crack tips of the casein-PAAm hydrogels, we tried to measure the fracture energies of casein-PAAm hydrogels. The work, $U$, done by the applied force to stretch the gel to a length $L$ was calculated by integrating the area under the load–extension curve in Figure S6. The work $U$ was then plotted against the notch length $c$ for the casein-PAAm hydrogels with different casein to casein plus acrylamide weight ratios. $L$ was defined as the length stretched when the notch turned into a running crack. In the present work, the notch became a running crack at the end of stretching and crack growth occurs just before the fracture of the notched gels. Thus, $L$ was directly determined as the increase in the length approaching the end of the stretching process of the gel. The fracture energy $\Gamma$ was calculated from eq 1 according to the method of Rivlin and Thomas (Figure 3c, d).

$$\Gamma = \frac{1}{b_0} \left( \frac{\partial U}{\partial c} \right)_L$$  

where $b_0$ is the initial thickness of the casein-PAAm hydrogel sample (3 mm) and $\frac{\partial U}{\partial c}$ is the slope of the $U$ vs $c$ curve at specified $L$. Calculated fracture energies increased drastically at 32 wt % casein because of its high stretchability and notch-insensitivity. The specific values of $L$ for various $\alpha$ are shown in Figure S6.

We suggest that the internal structure and intermolecular behaviors of casein-PAAm hydrogels (Figure 4a) are responsible for the high toughness and notch-insensitivity of casein-PAAm hydrogels. To investigate possible cross-links between the two types of polymers, we analyzed the casein gel, polyacrylamide gel, and casein-PAAm hydrogels with various casein to (casein plus acrylamide) weight ratio, $\alpha$ (Figure S7).
using Fourier transform infrared (FT-IR) spectroscopy. The absence of new peaks in the FT-IR spectra of casein-PAAm hydrogels suggested that there was no strong intermolecular covalent bonding between the PAAm chains and casein micelles. However, hydrogen bonding might exist between two networks. In the casein-PAAm hydrogels, the casein micelles are integrated into the PAAm network. The acrylamide chains form covalent cross-links with MBAA, and the casein micelles agglomerated into the 3D structure by hydrophobic associations. When a deformation was applied to the casein-PAAm hydrogels, friction between casein micelles and a plastic deformation of the casein micelle dissipated the energy (Figure 4b).

To demonstrate the dissipation mechanism of the casein micelles, we changed the amount of casein in the casein-PAAm hydrogels at a constant PAAm concentration (Figure 4c). We fixed the amount of PAAm at 7.5 wt %, and the amount of casein was increased from 0 wt % to 6 wt %. As shown in Figure 4c, the stretchability of the casein-PAAm hydrogel dramatically increased between 2 and 4 wt % casein in the gel. This indicated that the amount of casein highly affected the cross-linking density and the amount of energy dissipated. We believe there was a threshold amount of casein to obtain enough...
energy dissipation between casein micelles. As the size of a casein micelle is approximately 150 nm (Figure 4d), the intermicelle distance highly affects the bonding density and the total bonding energy. We carried out a rheological characterization to investigate the viscoelastic properties of the gels (Figure S8). The loss modulus of the casein hydrogel was relatively higher than that of the PAAm hydrogel. This implies that the casein micelle behaved plastically and dissipated energy in the hybrid gels. Additionally, the fracture energy of the casein-PAAm hydrogels decreased at high stretching rates (Figure S9). This result suggested that the area of the energy dissipating zone around the crack tip shrank under rapid deformation. The casein-PAAm hydrogels dissipated energy effectively, as shown by the hysteresis (Figure 4e). The casein-PAAm hydrogel displayed stress softening at the second loading. Stress softening on reloading indicates that energy was dissipated and that casein micelles were plastically deformed during the first tensile cycle. However, rupture stretch did not change, although the gels were unloaded from a stretch of 7 and reloaded immediately (Figure 4f). This meant that the flaw sensitivity of the gel was not changed by cycling.

In summary, we present a simple method to synthesize highly stretchable, notch-insensitive and tough casein-PAAm hydrogels. Casein-PAAm hydrogel was formed by the acidification of casein micelles and UV cross-linking of acrylamide. Although pure casein and pure PAAm hydrogels fractured in a brittle manner, the casein-PAAm hydrogels can be deformed and stretched over 35 times their original length and exhibited a high fracture energy (≈3000 J/m²). We suggest that the shape retention of the acrylamide network and the energy dissipation of casein micelles contributed to these enhanced mechanical properties of the gels. The energy dissipation could occur by two mechanisms: friction between casein micelles and plastic deformation of casein submicelles. This novel energy dissipation mechanism provides a better understanding of how to design a tough hydrogel. Thus, it can broaden current hydrogel studies and applications.

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**REFERENCES**


**ASSOCIATED CONTENT**

* Supporting Information
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Materials, detailed synthetic processes, mechanical tests, and scanning electron microscope (SEM) observation, swelling experiments in DI water and phosphate-buffered saline (PBS), transmission electron microscope (TEM) observation, FT-IR spectroscopy, and rheological characterization of the gels (PDF)

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**Notes**

The authors declare no competing financial interest.


