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Journal:	<i>RSC Advances</i>
Manuscript ID	RA-ART-11-2015-024414.R1
Article Type:	Paper
Date Submitted by the Author:	21-Dec-2015
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Subject area & keyword:	Gels & soft matter < Materials

Highly stretchable and tough pH-sensitive hydrogels with reversible swelling and recoverable deformation

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Abstract

Stimuli-responsive hydrogels are becoming increasingly important for controlled drug delivery, biosensing, and tissue engineering. However, few pH-sensitive hydrogels with good mechanical property have been extensively reported. In this investigation, we reported the preparation of extremely stretchable and tough pH-sensitive hydrogels by introducing a linear polysaccharide-agar into poly(acrylamide-co-acrylic acid) (P(AM-co-AA)) network and using diacrylated PEG (PEGDA) as a chemical crosslinker based on a simultaneous interpenetrating polymer networks (IPN) structure. It was found that longer PEGDA chains would lead to the obvious increase in swelling ratio and the stretchability of hydrogels. Moreover, the content of agar, the crosslinker concentration and the pH of external environment would significantly affect the mechanical characteristics of hydrogels. The compression strength and dissipated energy could reach 4.56 MPa and 0.56 MJ/m³ under the deformation of 85%, respectively. Also, hydrogels exhibited excellent reversible swelling and recoverable deformation. The improved mechanical property enables the pH-sensitive hydrogels to be potential candidates in the field of load-bearing soft tissues.

Keywords: hydrogels, agar, interpenetrating polymer network, pH-sensitive, reversible swelling, recoverable deformation

Introduction

Hydrogels, which were derived from natural and/or synthetic polymers, composed of three-dimensional, hydrophilic, polymeric networks could absorb and retain large amounts of water. Up to now, hydrogels as a ubiquitous tool had received considerable interest in regenerative medicine ¹, tissue engineering ^{2,3}, biomedicine ^{4,5}, actuators ⁶ and sensors ^{7,8}.

“Stimuli-responsive” or “smart” hydrogels could undergo significant volume/shape change depending on environmental conditions, such as pH ⁹, light ¹⁰, temperature ¹¹ and electric currents ¹². Among all intelligent hydrogels, pH-sensitive systems had been investigated intensively due to their potential usage in drug delivery system ^{4,5} or gene releasing ¹³. As a multifunctional soft and wet platform, the actual application of conventional hydrogels with weak mechanical properties had been seriously hindered due to their uneven intrinsic structure or lacking effective energy dissipation mechanism ¹⁴. Therefore, it would be much advantageous for intelligent hydrogels if they exhibit superior mechanical performances. Thus, developing high-strength hydrogels with reversible intelligent behavior is of both theoretical and practical significance.

A large number of strategies have been explored to enhance the strength or toughness of hydrogels, including double network (DN) hydrogels, nanocomposite hydrogels, slide-ring hydrogels, hydrophobic modified hydrogels, tetra-PEG gels and macromolecular microsphere composite (MMC) hydrogels ¹⁵⁻²⁰. Among them, the DN hydrogel was a typical sequential interpenetrating polymer network (IPN) ²¹, which

was often utilized to enhance, control, and/or combine functional properties^{15,22}. IPN hydrogels based on interpenetrating physically and chemically crosslinked networks had demonstrated some prominent mechanical properties²³⁻²⁵.

Agar is a linear polysaccharide extracted from marine red algae and consists of β -1,3 linked D-galactose and α -1,4 linked 3,6-anhydro- α L-galactose residues. Agar could form a gel when a homogeneous solution was cooled to a temperature of around 35°C, while translated to a sol at higher temperature above its melting point. The unique sol-gel phase transition of agar was thermoreversible due to the coil-helix structural transition²⁵⁻²⁷.

Poly(ethylene glycol) (PEG) and PEG-based materials had been considered for many applications because of their unique amphiphilic nature, biocompatibility, and polymerization characteristics²⁸⁻³⁰. PEG can be chemically cross-linked into hydrogels under mild polymerization conditions for a variety of applications. PEG modified with polymerizable end groups underwent very easy polymerization in water³¹. Acrylates were chosen as end groups because they could perform free-radical polymerization when used under the appropriate initiating condition³².

In this work, we aimed to construct a robust pH-sensitive hydrogel based on simultaneous IPN²¹. We engineered agar component with a thermoreversible sol-gel phase transition into a chemically crosslinked poly(acrylamide-co-acrylic acid) (P(AM-co-AA)) network to form a tough structure. Here, we chose hydrophilicity PEG diacrylate (PEGDA) as a chemical crosslinker. The pH sensitiveness and mechanical properties of hydrogels were investigated under the different conditions. It

is foreseen that the tough smart hydrogels will make a critical difference in meeting societal and industrial challenges.

Experiment

Materials

Polyethylene glycols (PEG) with molecular weights 2000 (PEG2K), 4000(PEG4K), 6000 (PEG6K) were supplied from Aladdin Chemical Reagent Co. Ltd., China and dried in a vacuum oven for 24 h before use. Dichloromethane and diethyl ether were provided by Guangfu Chemical Reagent Company, China. Triethylamine and acryloyl chloride were all purchased from Aladdin Reagent Co. Ltd., China. Acrylamide (AM), acrylic acid (AA) and agar (700-900 g/cm²) provided by Aladdin Reagent Co. Ltd., China were used to prepared hydrogels. N, N'-methylenebis (acrylamide) (MBA) as a cross-linker for hydrogels and potassium persulfate (KPS) as a thermal initiator for polymerization were received from Aladdin Chemical Reagent Co. Ltd., China. Sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium chloride (NaCl), used to control the pH and ionic strength of solution, were purchased from Beijing Chemical Works, China. All chemicals used were of analytical grade unless otherwise stated and used as received without further purification. All experiments were conducted in deionized water (diH₂O).

Preparation of PEG diacrylate (PEGDA)

The crosslinkers PEGDA with different molecular weights were prepared on the basis of a similar established literature procedure³³. Firstly, PEG was dissolved in methylene chloride in a 100mL round-bottomed flask. Then, two-fold molar excess triethylamine and acryloyl chloride were introduced dropwise into the reaction solution under nitrogen atmosphere, respectively. The final mixture was stirred at

room temperature for 24h under nitrogen, then the insoluble triethanolamine hydrochloride formed during the reaction were removed by filtration. The product was obtained in dry diethyl ether, filtered, and dried in a vacuum oven.

Hydrogel preparation

Agar/P(AM-co-AA) IPN hydrogels were synthesized by thermal initiated radical polymerization in diH₂O using PEGDA as a crosslinker and KPS as an initiator. The major procedure is described briefly as follows. As shown in Figure 1, firstly, a certain amount of Agar, AM (3.4mol L⁻¹) and AA (20wt% of AM) were dissolved in distilled water and the mixture was stirred for 30min at 90 °C until the solution became homogeneous. Then the crosslinking agents (MBA or PEGDA) and a thermal initiator (KPS) (0.5 mol% of monomers) were added into the solutions. After stirring under N₂ for several minutes, transparent solutions were obtained. Subsequently the solutions were injected into a simple reaction mold (90×60×3mm³), which were composed by a pair of glass plates and silica gel sidelines with 3.0 mm thickness. The P(AM-co-AA) network system was synthesized at 65 °C for 6h. Once formed, the P(AM-co-AA) hydrogels were cooled down at room temperature for 12h to form Agar/P(AM-co-AA) IPN hydrogels. The synthesized Agar/P(AM-co-AA) hydrogels were purified by immersing in diH₂O, which was refreshed daily, for at least 3d to remove any remaining un-reacted components and used for further mechanical tests. The Agar/PAM and P(AM-co-AA) hydrogels were also prepared at the same condition without adding AA or agar.

NMR characterization

About 5 mg of PEG or purified PEGDA was dissolved in 0.5 ml of DMSO-d₆. The chemical structures of F127 and F127DA were characterized by ¹H-NMR using a 400 MHz (AVANCE III 400 MHz, Bruker) spectrometer with DMSO-d₆ as the solvent. Measurements were performed at room temperature.

Swelling of hydrogels

Swelling tests were conducted to determine swelling property of the hydrogel by using a gravimetric method at room temperature. Approximately 0.15g dried hydrogel³⁴⁻³⁷ was immersed in buffer solutions with pH 2-8 (the ionic strength was adjusted to 0.1 M by addition of an appropriate amount of NaCl) or a methylene blue solution for 24h to ensure that the gels reached their maximum swollen state. The solution pH values were precisely measured by a pH meter. Then, the hydrogel was wiped with filter paper to remove excess water from the gel surface, and weighed carefully. The equilibrium swelling weights were obtained. The swelling ratio (SR) was calculated from the following equation:

$$SR = \frac{W_s}{W_d} \quad (1)$$

where SR is the swelling ratio, W_s and W_d stand for the weight of the swollen hydrogel and the weight of the air-dried hydrogel, respectively.

Reversible swelling

Reversible swelling tests were performed to study pH response reversibility of the hydrogel. The dried hydrogels were firstly placed in pH 2 buffer solution until there was no further weight change. Then, according to the report³⁸, the above hydrogels were immersed in pH 5.0 buffer solution until the hydrogel reached a constant weight

and subsequently transferred to pH 2.0 buffer solution, for four cycles. At a predetermined time, hydrogels were removed from the solutions and soaked up with filter paper to absorb excess water on surface, and then weighed. The swelling ratio (SR) was calculated by the dynamic weight change of the hydrogels with respect to time using the equation (1).

Mechanical measurements

The tensile and compression tests of the hydrogels were carried out on a tensile tester (SHIMADZU AGS-X 10KN) at room temperature. Before testing, hydrogels were fully equilibrated in buffer solutions (pH=1.0, 2.0, 5.0)³⁹. For tensile measurement, the samples were cut into a dumbbell shape with a gauge length of 30 mm, a width of 4 mm, and a thickness of 3 mm, and the stretching rate during the experiment was a constant velocity of 40 mm min⁻¹. The fracture strain, fracture stress and extension work were the corresponding values of the point at which the sample breaks. The dissipated energy was determined from loading-unloading test. For compression measurement, the samples were cylindrical (thickness: 15 mm; diameter: 32 mm). The compression rate during the experiment was a constant velocity of 2 mm min⁻¹.

Dynamic rheological measurements were performed by using a physical MCR 2000 rheometer (AR 2000ex, TA Instruments) between the parallel plates (25mm diameter) at room temperature. For frequency sweeping tests, storage modulus G' and loss modulus G'' were monitored within an angular frequency range of 0.1 to 100 rad/s at the strain of 0.5%.

Results and discussion

Choose of crosslinkers

Water-soluble PEGDA had been widely used in the synthesis of hydrogels and PEGDA acting as a chemical crosslinker could improve not only the hydrophilic ability in an aqueous medium, but also the biocompatibility of hydrogels³⁹⁻⁴¹. MBA was also used as small molecular crosslinker for the radical polymerization of acrylic monomers. Here, we compared the effect of MBA and PEGDA on the properties of hydrogels. PEGDA was synthesized by acylation reaction of the end hydroxyl groups of PEG with acryloyl chloride, as confirmed by ¹H NMR (DMSO-d₆). Figure S1, Supporting Information, showed the typical ¹H-NMR spectra of PEG6K and PEGDA6K. The peaks of double bonds from PEGDA6K appeared at 5.8-6.5 ppm⁴⁰.

The swelling characteristic is one of the most important factors on influencing the properties of hydrogels. Figure 2(a) showed the swelling behaviors of the Agar/P(AM-co-AA) IPN hydrogels with different crosslinkers in the methylene blue solution at room temperature. It was found that the PEGDA-crosslinked hydrogels had higher degree of swelling. The swelling ratio was closely correlated with the crosslinking density of hydrogels. The lower crosslinking density would result in the higher swelling ratio^{42,43}. The crosslinking density decreased with increasing the PEGDA molecular weights, resulting in the higher degree of swelling. Obviously, the wetting ability of Agar/P(AM-co-AA) hydrogel, which was crosslinked with higher molecular weights of PEGDA, was considerably enhanced.

Subsequently, the effect of the crosslinker with different molecular weights on the tensile properties of Agar/P(AM-co-AA) IPN hydrogels was also investigated (Figure 2(b)). It was found that the fracture strain of PEGDA6K-crosslinked Agar/P(AM-co-AA) IPN hydrogels could reach 1456%. In contrast, the fracture strain of MBA-crosslinked Agar/P(AM-co-AA) IPN hydrogels was only 647%. But the elastic modulus showed a decreasing trend with the increase of crosslinker molecular weights. The longer PEGDA molecular chains led to the lower crosslinking density, indicating that each individual chain between neighboring crosslinking points became longer. As a result, the movement of molecular chains in hydrogels was improved when the samples were stretched, and subsequently the fracture strain would increase. Moreover, it is evident that the PEGDA6K-crosslinked Agar/P(AM-co-AA) hydrogels become more flexible and extensible. In the following experiment, we choose PEGDA6K as a crosslinker to prepare the Agar/P(AM-co-AA) IPN hydrogels.

pH sensitiveness of hydrogels

Then we investigated whether the incorporation of AA and PEGDA would affect the pH-sensitive behavior of hydrogels. Figure 3(a) showed the swelling behaviors of hydrogels in solutions with different pH values ranging from 2.0 to 8.0 at room temperature. Clearly, the pH sensitiveness of hydrogels could not be affected by introducing PEGDA as a crosslinker, but the swelling ratio was significantly influenced. It was found that the hydrogel without acrylic acid groups did not show any pH sensitiveness and the incorporation of AA could remarkably improve the pH sensitiveness of hydrogels. The swelling ratios showed significant difference between

acidic and neutral environment. The hydrogels exhibited lower swelling ratios and the swelling ratios changed little at pH ranging from 2.0 to 4.0. While in a neutral medium, the gel became swollen. As shown in Figure S2, Supporting Information, in a strong acidic medium, the carboxylate anions in hydrogels were mostly protonated. So the swelling ratios decreased due to the reinforced hydrogen-bonding interaction among carboxylate groups and the restricted electrostatic repulsion among carboxylate groups. With the increase of pH, some of the carboxylate groups were ionized, causing the breakage of hydrogen-bonding interaction and the enhanced electrostatic repulsion between anionic carboxyl acid groups. Consequently, the hydrogels tended to be well swollen^{44,45}. It was also obvious that Agar/P(AM-co-AA) IPN hydrogels showed good reversible swelling in solutions with different pH (Figure 3(b)).

We also found that the mechanical properties of Agar/P(AM-co-AA) IPN hydrogels were closely related to the solution pH. The result data was given in Table 1 and the corresponding tensile curves were shown in Figure S3, Supporting Information. There was no obvious change for the tensile strength and work of Agar/PAM hydrogels when the pH value was increased from 2.0 to 5.0. However, the mechanical properties of Agar/P(AM-co-AA) IPN hydrogels exhibited a severe decrease trend. At a lower pH, the hydrogen bond interactions between the protonated carboxylic acid groups of hydrogels were strong, resulting in a tight network. When the pH value was increased, more hydrogen bonds were damaged and the electrostatic repulsion increased. Hence, the hydrogels became more swollen and more serious deterioration of the mechanical

properties occurs. These results offered further evidence that hydrogen bond was responsible for enhancing the strength of hydrogels³⁹. It was a remarkable fact that the properties of Agar/P(AM-co-AA) IPN hydrogels could be adjusted depending on the environmental pH.

Mechanical properties of hydrogels

The mechanical properties of hydrogels are extremely crucial for their practical application. As shown in Figure 4, PEGDA6K-crosslinked Agar/P(AM-co-AA) IPN hydrogels exhibited an extraordinary mechanical property. The hydrogels could easily withstand high-level deformations including knotting (Figure 4a,b), large stretching (Figure 4c), entanglement (Figure 4d), bending (Figure 4e) and compression (Figure 4f) without any obvious damage. It was notable that the hydrogels were able to regain their initial shape after unloading, indicating that the hydrogels had a certain shape-recovery property.

The compression loading-unloading curves and dissipated energy of hydrogels were shown in Figure 5. The area of loading and unloading closed curves reflected the dissipated energy per unit volume^{24,47}. Clearly, the hydrogels were capable of sustaining a compression strain of over 85% with full strain recovery (Figure 5a). It was found that the compression strength and corresponding dissipated energy of P(AM-co-AA) hydrogel was only 275 kPa and 0.047 MJ/m³ at the strain of 85%. In contrast, the compression strength and dissipated energy of Agar/ P(AM-co-AA) IPN hydrogel could reach 4.56 MPa and 0.56 MJ/m³ (Figure 5b). This was due to the efficient energy dissipation provided by physically linking agar helix bundles. It was

also found that the compression strength and corresponding dissipated energy decreased slightly during four successive compression cycling test of Agar/P(AM-co-AA) IPN hydrogel under 85% strain (Figure 5c).

Agar/P(AM-co-AA) hydrogels also displayed much better tensile properties than P(AM-co-AA) hydrogels (Figure 6). Agar/P(AM-co-AA) hydrogels could achieve a tensile strength of 182 KPa and dissipated energy of 1.22 MJ/m^3 , which was superior to that of P(AM-co-AA) hydrogels (tensile strength 51KPa, dissipated energy 0.27 MJ/m^3) under 800% strain. The agar network was formed by physically linking agar helix bundles. The incorporation of the agar network provided the efficient energy dissipation and made the internal structure in the system more homogeneous. These results indicated that the agar network could significantly improve the mechanical strength of the hydrogels. A series of tensile tests were conducted to examine the tensile properties of Agar/P(AM-co-AA) hydrogels in response to the changes in PEGDA6K and agar concentration. The results are discussed and summarized in Figure S4 and S5, Supporting Information.

The energy dissipation which could be characterized by hysteresis was also an important feature of tough hydrogels⁴⁸. The loading-unloading tests were performed to evaluate the energy dissipation of Agar/P(AM-co-AA) IPN hydrogels. Figure 7(a) illustrated tensile cycling curves of Agar/P(AM-co-AA) hydrogels at different strains. Pronounced hysteresis loops were observed on tensile cycling curves, indicating that the Agar/P(AM-co-AA) hydrogels dissipated energy effectively. Moreover, Figure 7(b)

showed that the hydrogels could dissipate energy as much as 2.4 MJ/m^3 at the strain 1000%.

Based on the fact that agar could melt to sols upon heating to $90\text{-}95 \text{ }^\circ\text{C}$ ²⁶, we performed the tensile cycling tests to examine time-dependent recovery of Agar/P(AM-co-AA) IPN hydrogels. The hydrogels were stored at $100 \text{ }^\circ\text{C}$ above the melting point of the agar for some time after the first loading-unloading cycle. Figure 8(b) showed the recovery of dissipated energies that were calculated from Figure 8(a). The tensile cycling curve achieved 66% recovery of hysteresis area after the hydrogel was soaked in water at $100 \text{ }^\circ\text{C}$ for 30 min. After increasing the heating time from 30min to 60min, the recovered dissipated energy increased considerably from 66% to 89% of its original value. These results markedly showed that the hydrogel network could be much better healed via a thermo sol-gel transition²⁵. When the hydrogel (Figure S6a, Supporting Information) was stretched to a previously definitive deformation, the agar helix bundles ruptured into small clusters (Figure S6b, Supporting Information) and dissipated energy. The small clusters transformed into linear conformation when stored at a temperature above the melting point. As a result, the linear agars interpenetrated into P(AM-co-AA) network (Figure S6c, Supporting Information). Subsequently, the linear agars reformed a double-helix structure and aggregated into helical bundles during a cooling process, leading to the recovery of hydrogels (Figure S6a, Supporting Information)²⁵.

The rheology property of hydrogels with different crosslinkers was measured by frequency sweeps and shown in Figure 9. Both hydrogels exhibited a predominantly

elastic characteristics, with the elastic (storage) modulus G' exceeding their corresponding viscous (loss) modulus G'' over the whole frequency range⁴⁹. Both G' and G'' of the MBA crosslinked hydrogels were higher than that of the PEGDA6K crosslinked hydrogels. That is, the longer PEGDA molecular chains led to the lower crosslinking density, leading to a more flexible structure. Figure S7, Supporting Information, displayed the effect of agar content on the rheological properties. It was clear that introducing agar had an obvious effect on the G' which was a good parameter to measure the material stiffness⁵⁰ and the G' and G'' significantly increased with the increase of agar content in hydrogels. It was also found that both G' and G'' were almost frequency-independent within the frequency region of 0.1-100 rad/s, indicating the formation of a rigid network structure⁵¹. The crosslinking density mainly determined the stiffness of the hydrogel and the crosslinking density of hydrogel network increased with the increase of agar content. The results were consistent with the mechanical properties of hydrogels in Figure S4.

Conclusions

In summary, highly stretchable, tough and pH-sensitive Agar/P(AM-co-AA) IPN hydrogels were successfully prepared by introducing agar components into a chemically crosslinked P(AM-co-AA) networks. PEGDA crosslinked hydrogels showed an obvious increase of swelling ratio and stretchability. Compared to P(AM-co-AA) hydrogels, Agar/P(AM-co-AA) IPN hydrogels were extensible and effectively dissipated energy by an obvious hysteresis during loading-unloading process. Moreover, the hydrogels exhibited remarkable deformation recovery and reversible swelling behaviors. The mechanical characteristics of Agar/P(AM-co-AA) IPN hydrogels could be changed by the pH of media. The pH-sensitive hydrogels with toughness, stretchability and deformation recoverability would extend soft materials as smart candidates in biomedical applications.

Acknowledgements

This research was supported by a grant from National Natural Science Foundation of China (NSFC) (No. 51473023 and 51103014).

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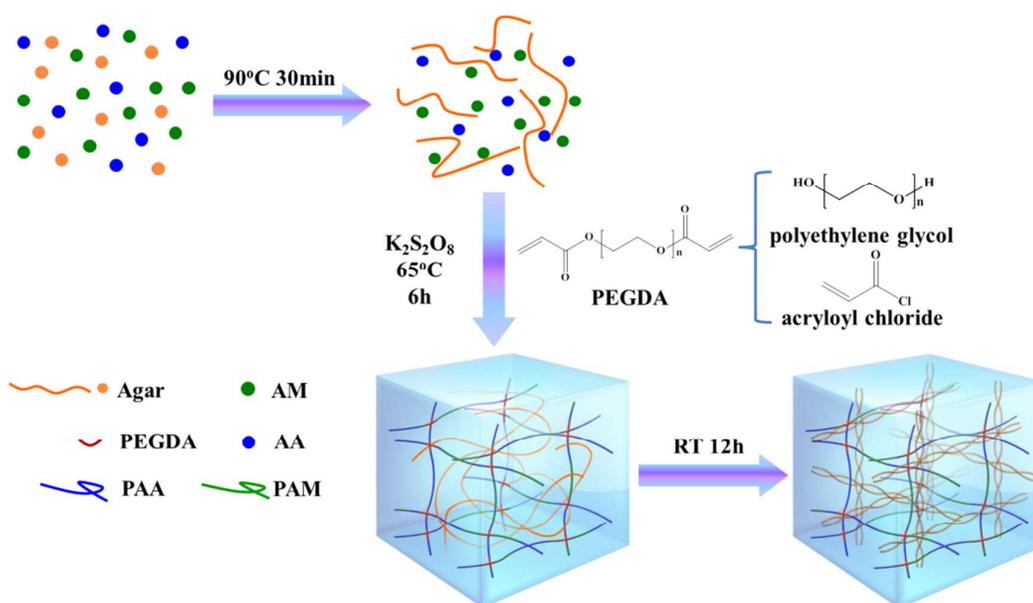


Figure 1 Schematic illustration of the preparation of Agar/P(AM-co-AA) IPN hydrogels.

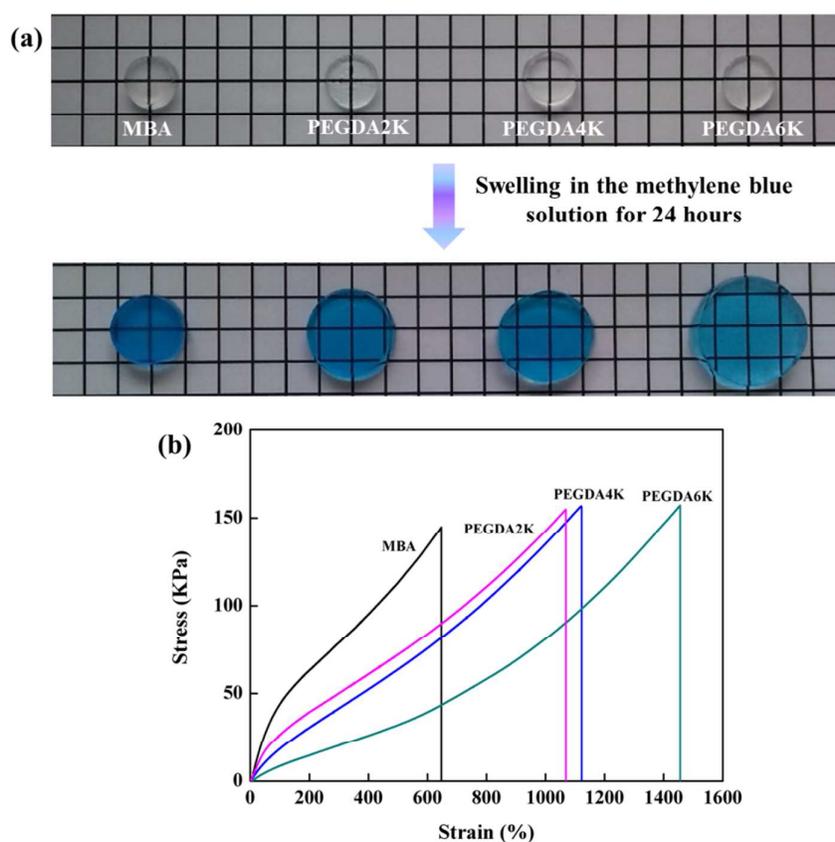


Figure 2(a) Swelling behavior of Agar/P(AM-co-AA) IPN hydrogels with different crosslinkers; (b) Tensile properties of Agar/P(AM-co-AA) IPN hydrogels with different crosslinkers.

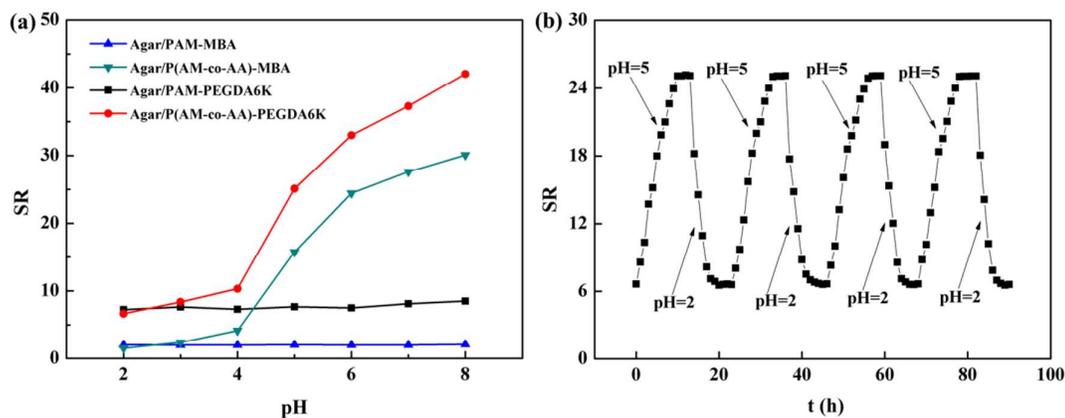


Figure 3 (a) Swelling ratio as a function of pH for Agar/PAM and Agar/P(AM-co-AA) IPN hydrogels; (b) Reversible swelling of Agar/P(AM-co-AA) IPN hydrogels at pH 2 and pH 5.

Table 1. Dependence of mechanical properties on pH.

Hydrogel	Crosslinker	Fracture stress		Fracture strain		Work		Elastic		SR	
		[kPa]		[%]		[MJ/m ³]		modulus [kPa]			
		pH=2.0	pH=5.0	pH=2.0	pH=5.0	pH=2.0	pH=5.0	pH=2.0	pH=5.0	pH=2.0	pH=5.0
Agar/PAM	PEGDA6K	145.6	141.9	1535	1572	1.07	1.053	38.17	35.69	7.24	7.66
Agar/P(AM-co-AA)	PEGDA6K	152.7	51.6	1412	295	1.02	0.089	60.97	48.77	6.65	25.09

The work of extension, which is the total work required for the fracture of a unit volume of a material and defined as the area under the tensile stress-strain curves, was used as the index of toughness[46].

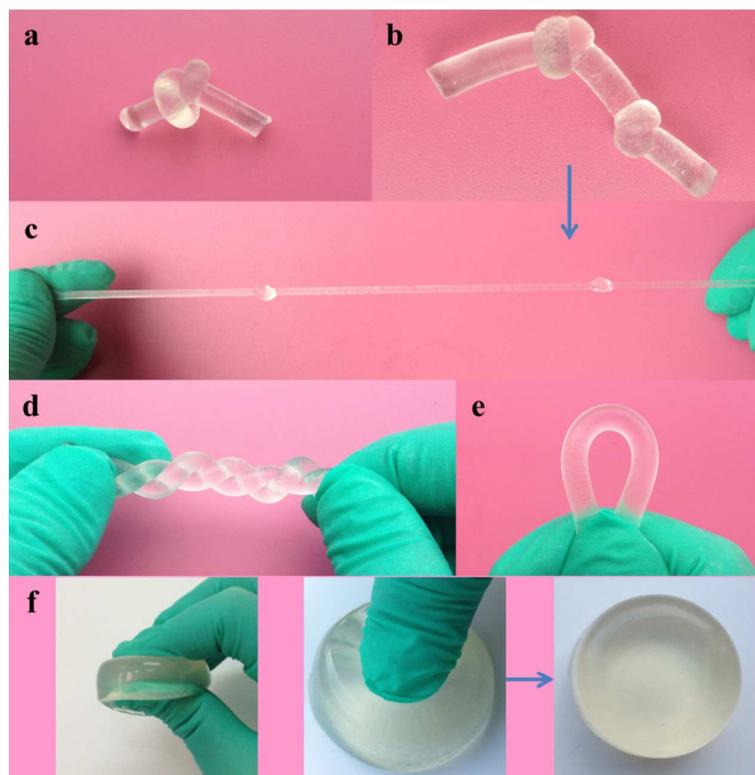


Figure 4 Photographs of Agar/P(AM-co-AA) IPN hydrogels with extraordinary mechanical properties: (a, b) The hydrogel is tied into one or more knots; (c) The hydrogel is stretched; (d) Two hydrogels are entangled together; (e) The hydrogel is bended; (f) The hydrogel is compressed.

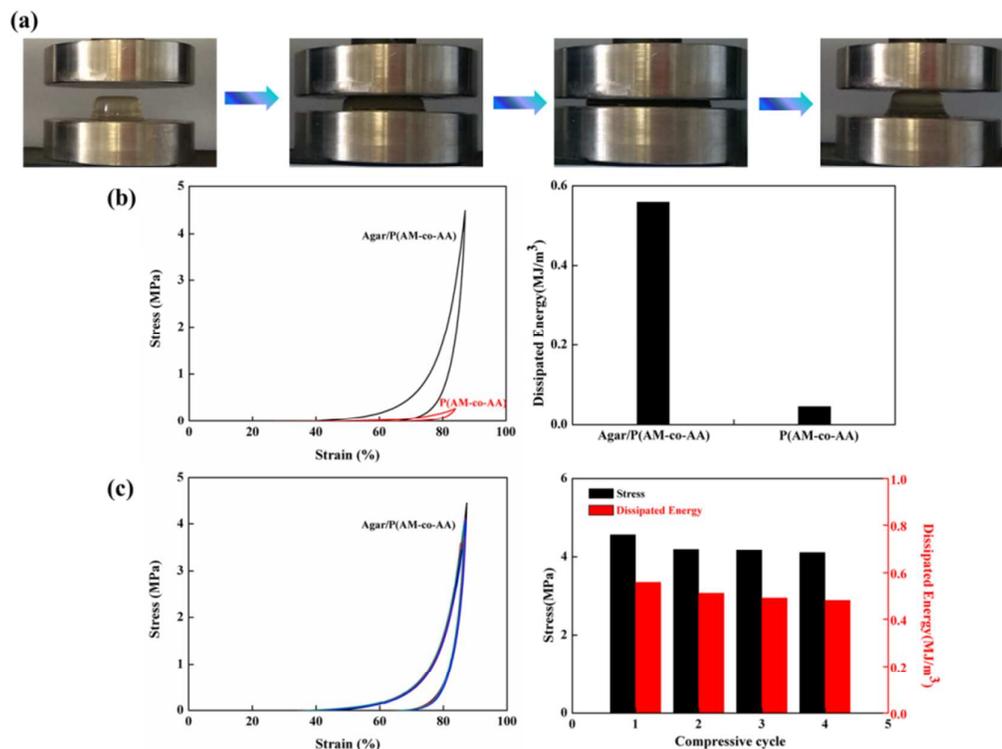


Figure 5 Compression properties of Agar/P(AM-co-AA) and P(AM-co-AA) hydrogels. (a) Images of Agar/P(AM-co-AA) hydrogels undergoing compression testing to 85% strain; (b) Compression cycling tests of Agar/P(AM-co-AA) and P(AM-co-AA) hydrogels under 85% strain and the corresponding dissipated energy; (c) Four successive compression cycling tests of Agar/P(AM-co-AA) hydrogels under 85% strain and the corresponding stress and dissipated energy.

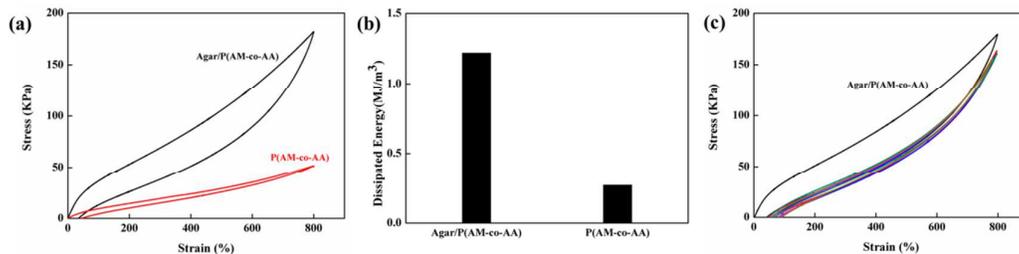


Figure 6 Tensile properties of Agar/P(AM-co-AA) and P(AM-co-AA) hydrogels. (a) Tensile cycling tests of Agar/P(AM-co-AA) and P(AM-co-AA) hydrogels under 800% strain; (b) The corresponding dissipated energy; (c) Successive tensile cycling tests of Agar/P(AM-co-AA) hydrogels under 800% strain.

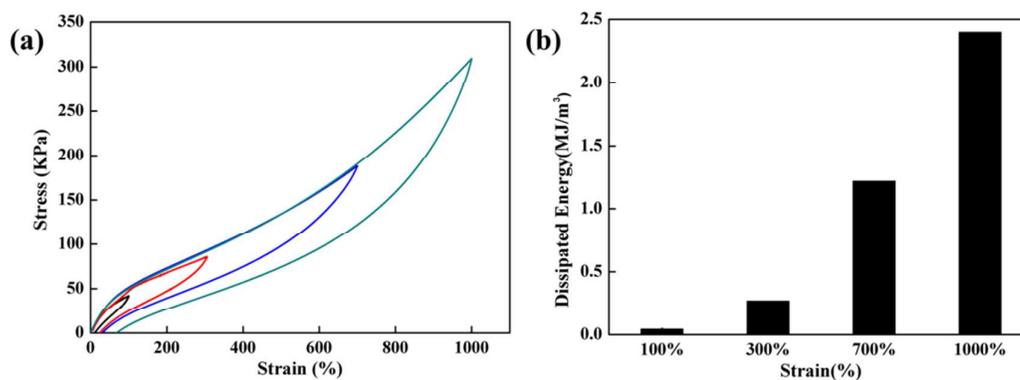


Figure 7 Tensile cycling tests of Agar/P(AM-co-AA) IPN hydrogels. (a) Samples were subjected to previously definitive strain (100%, 300%, 700%, 1000%) and then unloaded to zero; (b) The corresponding dissipated energy of Agar/P(AM-co-AA) hydrogels.

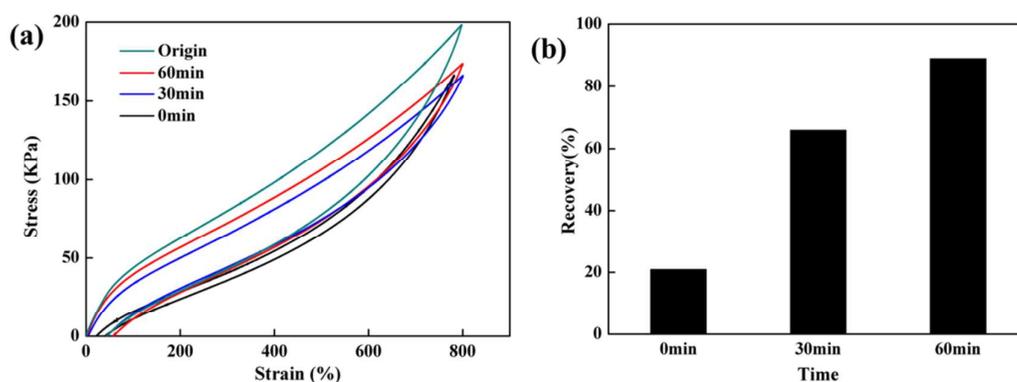


Figure 8 (a) Recovery tests of Agar/P(AM-co-AA) IPN hydrogels stored at 100 °C; (b) The time-dependent recovery of corresponding dissipated energy.

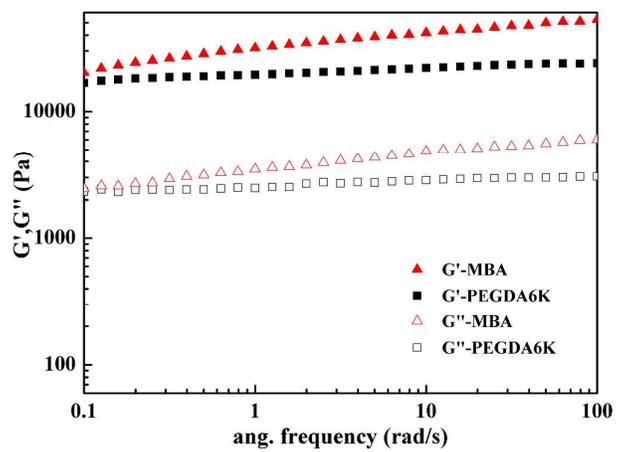


Figure 9 Rheological properties as a function of frequency at strain of 0.5% for the hydrogels crosslinked with MBA or PEGDA6K at 25°C.