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Preparation and properties of a novel thermo-responsive poly(*N*-isopropylacrylamide) hydrogel containing glycyrrhetinic acid

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Abstract A novel thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel containing glycyrrhetinic acid(GA) was synthesized by free radical copolymerization. The structure of the product was confirmed by FT-IR and ^{1}H-NMR spectra. The temperature responsibility and swelling properties of the copolymerized hydrogel were investigated by differential scanning calorimetry (DSC) and gravimetric methods. The results indicated that GA-incorporated hydrogel was still temperature responsive and the swelling ratio decreased with the increasing of temperature. The lower critical solution temperature (LCST) of GA-incorporated hydrogel and poly(*N*-isopropylacrylamide) hydrogel was 30.00 °C and 31.21 °C, respectively, in distilled water. However, these two values were shifted to 28.22 °C and 29.16 °C in cell culture media. The novel hydrogel also exhibited reversible temperature responsibility. Deswelling kinetics indicated that the copolymerized hydrogel deswelled more rapidly than poly(*N*-isopropylacrylamide) hydrogel. Since GA has specific binding capacity to asialoglycoprotein receptors on the membrane of hepatocyte, this novel hydrogel with GA could be expected as good candidate for hepatic cell culture.

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Introduction

Intelligent hydrogels, due to their unique environmental responsiveness, have been widely applied in the fields of tissue engineering, drug delivery system, biological separation, enzyme immobilization, and signal sensor [1–4]. As a kind of typical temperature-responsive hydrogel, the shape, volume, and the swelling ratio of PNIPAAm show dramatic changes upon reaching its LCST (32 °C) in aqueous solution. It absorbs water to a swollen state at a temperature below the LCST, and shrinks with an abrupt volume decrease when the temperature goes above the LCST because of the rapid alteration in hydrophilicity and hydrophobicity [5]. The temperature responsiveness of PNIPAAm has been used in detachment of intact cell sheet. Cells secrete extracellular matrix (ECM) molecules and form cell-to-cell junctions in cell culture process. The typical method of cell recovery after the formation of cell sheet is proteolytic digestion by trypsinization. Both ECM and cell-to-cell junction proteins are destroyed by this way which make it very difficult to obtain the intact cell sheet [6]. Using cell culture plate covered or modified by temperature-responsive hydrogel can avoid the use of proteolytic enzymes. When temperature is higher than LCST of PNIPAAm, it becomes slightly hydrophobic, allowing various types of cells to proliferate and spread on the surface of plate. When temperature is lower than LCST, it will undergo a spontaneous change and become hydrophilic. Under low temperature conditions, a hydration layer is formed between the supporter and attached cells, allowing for the harvest of confluently cultured cells as intact sheets [7]. However, the poor biocompatibility of PNIPAAm often results in a very low survival rate of the cells. In order to exploit the potential applications of PNIPAAm hydrogels, much attention has been paid to the

synthesis of copolymerized PNIPAAm hydrogels with unique properties such as good biocompatibility, biodegradability, and biological functionality [8–12]. For example, a number of polysaccharides, such as chitosan, alginate, cellulose, and dextran, have been used to combine with PNIPAAm to form biological intelligent gels, which can be widely used in the fields of tissue engineering, controlled drug release, etc [13–18].

GA is one of the effective components in Chinese medicine liquorice. The existence of specific receptor of GA on the surface of hepatic cell membrane has been proved [19, 20]. Liposome modified by GA has very good targeting property toward hepatic cell. Therefore, most of the studies about GA were focused on the hepatic targeted drug delivery system (HTDDS) [21, 22]. However, there were no related reports on using GA in the hepatic cell culture until now.

In the present study, GA was introduced and poly(*N*-isopropylacrylamide) hydrogel containing glycyrrhetinic acid (P(NIPAAm-co-AAC-GA)) was prepared to improve the biocompatibility and the hepatic affinity of the traditional PNIPAAm hydrogel. The temperature responsive properties of the novel hydrogen were investigated. The result of this research will provide the basis for the further research about the hepatic cell culture.

Experiment

Materials

NIPAAm was obtained from Kohjin Co. (Japan) and purified by recrystallization from n-hexane. GA was purchased from Fujie medical Co. (Xi'an, China), fetal bovine serum (FBS) was the product of Minhai biological Co. (Beijing, China), Dulbecco's modified eagle's medium (DMEM, 10% FBS) was the product of HYCLONE Co. (Logan, Utah, US), *N*-hydroxysuccinimide (SuOH) and *N*,*N*-dicyclohexyl carbodiimide (DCC) were supplied by Sinopharm Chemical Reagent Co.(Shenyang, China). Ethylenediamine, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), *N*,*N*,*N*,*N*-tetramethylenediamine (TEMED), *N*,*N*-methylenebis(acrylamide) (MBAA), etc. were of analytical grade made in China.

Synthesis of GA amine derivative (GA-NH_2)

GA-NH_2 was synthesized according to the method of Yuan and coworkers [23]. Briefly, 2.895 g GA was dissolved in THF and cooled to −10 °C and then 1.77 g DCC was added to this solution and stirred for 30 min. SuOH (0.71 g, 6.15 mmol) was then added to the above solution and stirred at −10 °C for 3 h. Subsequent stirring was done at 20 °C for 18 h. After that, intermediate product dicyclohexylurea was excluded by filtration. The resultant filtrate was poured into aether to get white precipitate which was washed with aether and dried under vacuum to get glycyrrhetinic succinimide ester. Glycyrrhetinic succinimide ester (1.015 g) was dissolved in DMF (15 mL) and then dropped into 35 mL ethylenediamine. The reaction mixture was stirred for additional 24 h at 60 °C and then poured into distilled water under stirring for the formation of precipitate. The final product GA-NH_2 was obtained by filtration.

Synthesis of GA-AAC monomer

Five grams of GA-NH_2 was dissolved in 20 mL DMF, 1 mL AAC was then added to the above solution under the protection of nitrogen atmosphere. After that, 0.01 g sulfo-NHS and 0.07 g EDC were added to let the reaction continue for 24 h at room temperature. Intermediate product urea was eliminated by filtration. GA-AAC was obtained by dropping the filtrate into the distilled water and precipitating under stirring.

Synthesis of P(NIPAAm-co-AAC-GA) hydrogel

P(NIPAAm-co-AAC-GA) hydrogel was synthesized by free radical copolymerization of NIPAAm and GA-AAC in DMF solution using AIBN and TEMED as an initial system. Specially, GA-AAC and NIPAAm were dissolved in DMF according to a weight ratio *r*, in the feed (GA-AAC/NIPAAm) of 1:3, and then AIBN (0.2 wt%) and MBAA (2 wt%) were added. After bubbling with nitrogen gas to remove oxygen, TEMED (2 wt%) was added with stirring and the copolymerization was proceeded at 60 °C for 24 h. The copolymerized hydrogels were cut into thin disks of 24 mm in diameter and then immersed in distilled water for 1 week to remove any unreacted compounds. Scheme 1 represents the synthetic route of P(NIPAAm-co-AAC-GA) hydrogel.

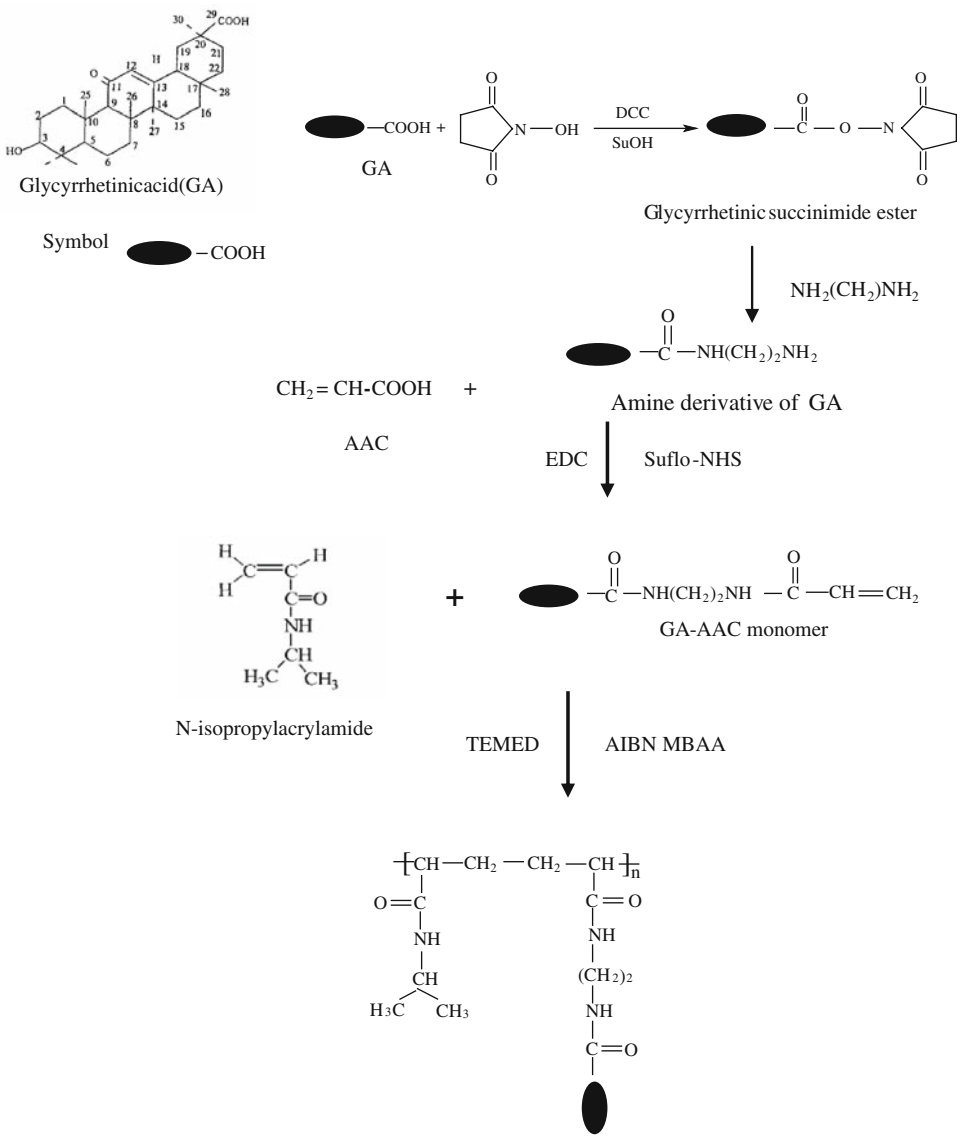
FT-IR and ^{1}H-NMR analysis

GA, GA-NH_2, GA-AAC, and dried P(NIPAAm-co-AAC-GA) hydrogels were powdered and ground with KBr and pressed into pellets under reduced pressure. Fourier transform infrared spectroscopy measurements were carried out (Vector 22 FTIR, BRUKER Co., Germany). ^{1}H-NMR measurements were conducted at room temperature using DMSO as solvent (Varian UNITY Plus-400 NMR, USA).

Thermal analysis of P(NIPAAm-co-AAC-GA) hydrogel

The LCSTs of P(NIPAAm-co-AAC-GA) and PNIPAAm hydrogel were determined using differential scanning

Scheme 1 Synthetic route of P(NIPAAm-co-AAC-GA) hydrogel



calorimetry (DSC7, PE company, USA). All hydrogels were dipped into distilled water and DMEM separately. For each DSC measurement, 10 mg equilibrium swollen sample was placed inside a hermetic aluminum pan and then sealed tightly by a hermetic aluminum lid. The thermal analysis was performed from 20 to 50 °C at an increasing rate of 2 °C/min under dry nitrogen.

Interior morphology of hydrogels

The morphology of the hydrogel cross sections was visualized by a scanning electron microscope (Quanta 200, USA). Hydrogel samples at their maximum swollen state were frozen in liquid nitrogen and freeze-dried in a desktop freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) under vacuum at −60 °C for 3 days until all water was sublimed. The freeze-dried samples were then fractured into smaller pieces with a sharp razor blade and then mounted on aluminum stubs with carbon black paste. All the samples were then vapor coated with gold in a sputter coating system.

Swelling behavior of hydrogels

The swelling ratios and swelling kinetics of hydrogel samples were measured gravimetrically. Weights of swollen hydrogels were obtained after wiping off the excess water on the surfaces with moistened filter paper. The average value of three measurements from three parallel specimens in the same hydrogel was taken for each sample.

Effect of temperature on equilibrium swelling ratio

The hydrogel samples were immersed in distilled water at a predetermined temperature ranging from 20 to 40 °C for at least 24 h. When the swelling equilibrium was reached, the

hydrogels were removed and weighted. After weight measurement at one temperature, the hydrogels were re-equilibrated at another predetermined temperature for subsequent measurement. The equilibrium swelling ratio was calculated as following equation.

$$\text{Swelling ratio (SR)} = W_s/W_d \quad (1)$$

where W_s is the weight of the equilibrated hydrogel at each temperature, and W_d is the dry weight of hydrogel.

Deswelling kinetics

The deswelling kinetics was measured at 40 °C. The hydrogel samples were first immersed in distilled water at 20 °C till equilibrium. The equilibrated hydrogel samples were then quickly transferred into a water bath at a temperature of 40 °C. At predetermined time intervals, the samples were taken out from the hot water and weighed after wiping off the excess water on the surface with filter papers. The water retention (WR) of the hydrogels was defined as the following Eq. 2

$$\text{WR} = [(W_t - W_d)/(W_s - W_d)] \times 100\% \quad (2)$$

where W_t is the weight of the wet hydrogel at particular time, W_s is the weight of the equilibrated hydrogel at 20 °C, and W_d is the dry weight of hydrogel.

Reswelling kinetics

The reswelling kinetics was determined by immersing the dry hydrogel in water at 20 °C. Samples were periodically removed and weighed during the reswelling process. The water uptake (WU) was defined as the following equation

$$\text{WU} = [(W_t - W_d)/W_d] \times 100\% \quad (3)$$

where W_t and W_d are the same as defined earlier in Eq. 2.

Swelling reversibility of hydrogels

The hydrogels equilibrated in water at 20 °C were quickly transferred into water at 40 °C. At specific time intervals, the hydrogels were taken out of water and weighed. After the weight of the samples become constant, they were transferred from water at 40 °C to water at 20 °C; the weight was then recorded at different times until they become stable. The measurement of deswelling and reswelling was repeated again. Furthermore, oscillatory swelling behavior over a shorter time interval was also investigated. The samples were placed in water at 40 °C and the weights of hydrogels with 3 min time intervals were recorded. After 12 min, the samples were transferred into water at 20 °C and were weighed every 3 min. The above process was repeated several times. The water retention of the hydrogels was calculated as above.

Results and discussion

FT-IR and ^{1}H-NMR analysis

To confirm the structure of all synthesized products, FT-IR and ^{1}H-NMR spectra measurements were carried out. Figure 1 illustrated the FT-IR spectra for GA, GA-NH_2, GA-AAC, P(NIPAAm-co-AAC-GA), and NIPAAm. The characteristic peak of carboxyl groups of GA at 1740–1690 cm^{-1} was absent from spectra of the derivatives of GA. The presence of characteristic peaks in the spectrum of GA-NH_2 at 1660–1640 and 1540 cm^{-1} was assigned to the amide I band (C=O stretching) and amide II band (N–H bending), respectively, which indicated the successful bonding of –NH_2 group to GA. In the spectrum of GA-AAC, the peak at 1656 cm^{-1} was assigned to amide I band, C=O stretching vibration peak produced by conjugation of C=C and C=O appeared at 1677 cm^{-1}, and the out-of-plane bending vibration of C–H of alkene appeared at 980 cm^{-1}, 920 cm^{-1}. This indicated that AAC was bond to –NH_2 group of GA-NH_2. Almost all the characteristic peaks of PNIPAAm and GA-AAC appeared in the spectrum of P(NIPAAm-co-AAC-GA). Furthermore, the disappearing of C=C twisting vibration peaks at 635–630 cm^{-1}, 552–500 cm^{-1} indicated that GA-AAC had been copolymerized with PNIPAAm.

The ^{1}H-NMR spectra of GA, GA-NH_2, and GA-AAC are shown in Fig. 2. In comparison with the ^{1}H-NMR spectrum of GA, the spectra of GA derivatives exhibited the peaks of protons in amide at 7.8–8.4 ppm instead of the characteristic resonance peak of the proton in carboxyl at 12.2 ppm. The peak at 7.8–8.4 ppm is multiple in the

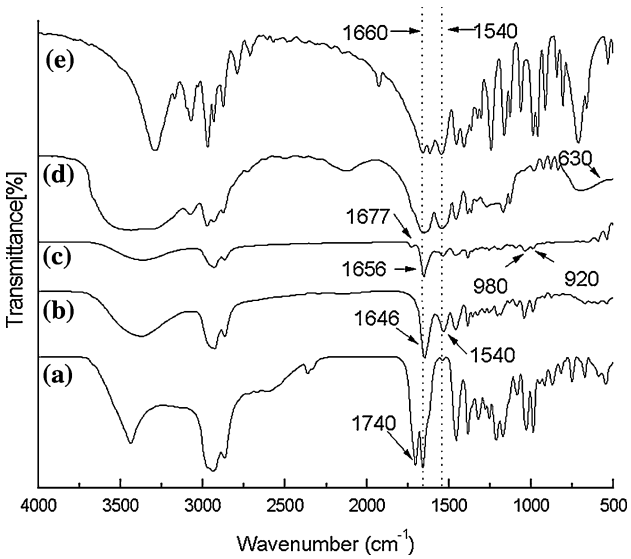


Fig. 1 FT-IR spectra for GA (*a*), GA-NH_2 (*b*), GA-AAC (*c*), P(NIPAAm-co-AAC-GA) (*d*), and PNIPAAm (*e*)

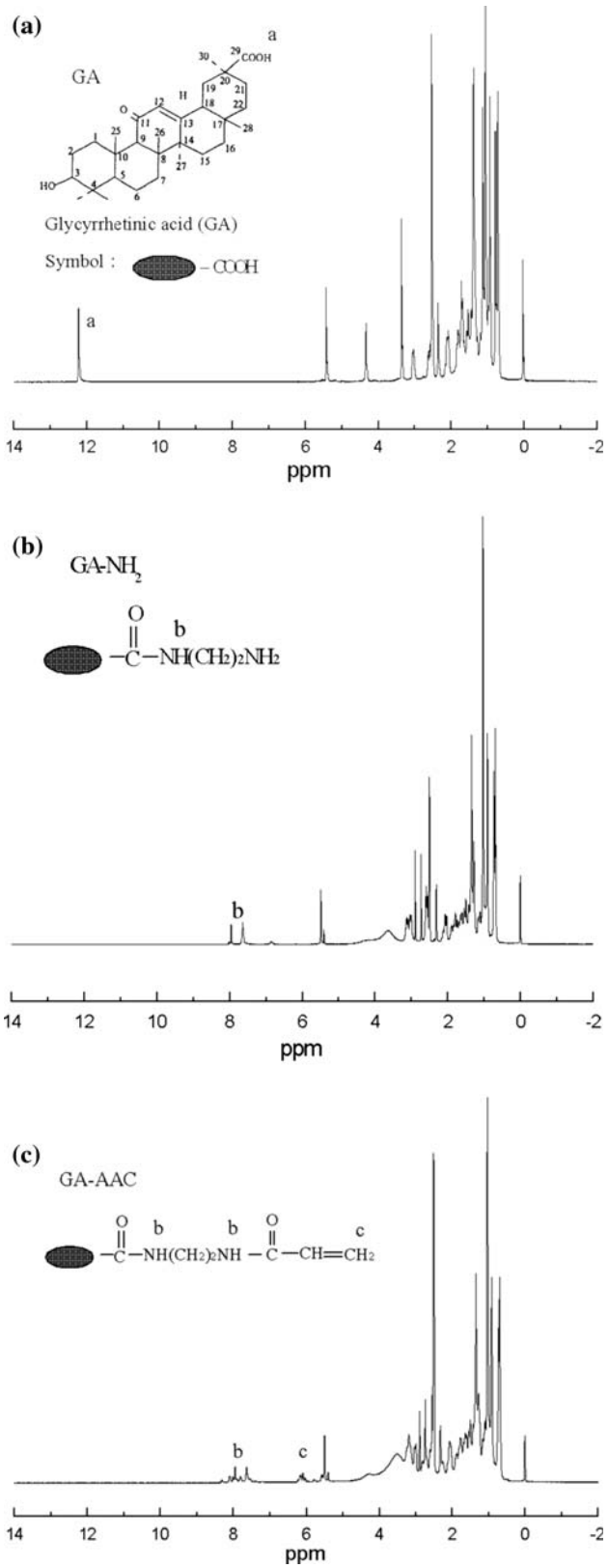


Fig. 2 ^{1}H-NMR spectra of GA (**a**), GA-NH_2 (**b**), and GA-AAC (**c**)

spectrum of GA-AAC due to the presence of two amide bonds in GA-AAC molecule. In addition, the vinyl proton peak at 6.0–7.5 ppm in spectrum of GA-AAC was not presented in the spectrum of GA-NH_2.

DSC thermograms of hydrogels

Considering that the synthesized hydrogels will be used in cell culture, the LCSTs of all hydrogels in distilled water and Dulbecco's modified eagle medium (DMEM) were investigated. DSC thermograms of these hydrogels in the temperature range 20–45 °C are shown in Fig. 3. The LCST was defined as the onset temperature of the endotherms as shown [24–26]. Figure 3 indicated that LCSTs of PNIPAAm hydrogel were 31.21 °C in distilled water and 29.16 °C in DMEM, respectively. However, these two values of P(NIPAAm-co-AAC-GA) hydrogel were shifted to 30.00 °C and 28.22 °C, respectively. P(NIPAAm-co-AAC-GA) hydrogel exhibited lower LCST than PNIPAAm hydrogel. It is known that there exists a hydrophilic/hydrophobic balance in the PNIPAAm network, and when the external temperature is above LCST, the strengthened hydrophobic interactions among the polymeric system would lead to phase separation. If the hydrophilic/hydrophobic balance was shifted toward a more hydrophobic nature, hydrogel will exhibit a lower LCST [27–29]. Here, large numbers of hydrophobic carbon rings in GA molecule made it exhibit hydrophobicity. The incorporation of the hydrophobic GA into P(NIPAAm-co-AAC-GA) resulted in

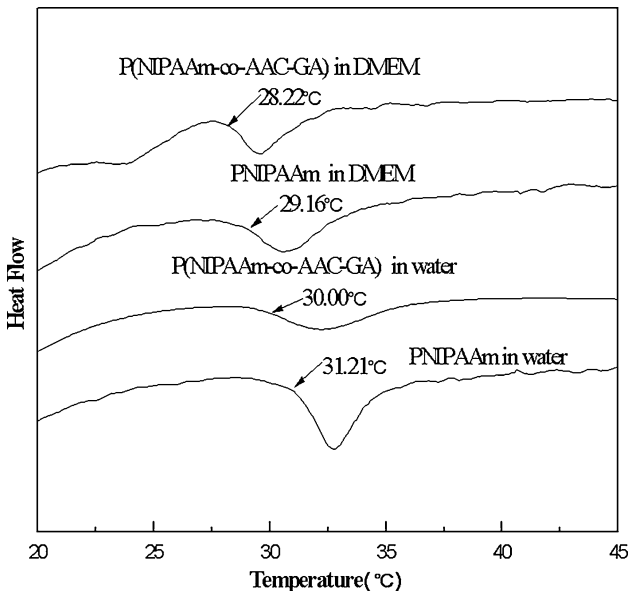


Fig. 3 LCST of P(NIPAAm-co-AAC-GA) gel and PNIPAAm gel in distilled water and in cell culture medium

decreased number of hydrogen bonds. Therefore, less energy was needed to destroy these hydrogen bonds and the LCST decreased accordingly. Besides, it is also shown in Fig. 3 that LCSTs of the two kinds of hydrogel in distilled water were higher than that in DMEM. The reason was that the existence of salts (NaCl, $NaHCO_3$), amino acid, and D-Glucose, etc. in DMEM weakened the strength of hydrogen bonds between hydrogel and water. From the result of DSC investigation, it can be concluded that the LCST of copolymerized hydrogel was between room temperature and normal body temperature, which was favorable for the transition of adhesion and detachment of cell by controlling temperature.

SEM micrographs of hydrogels

SEM images of the internal structure of PNIPAAm and P(NIPAAm-co-AAC-GA) hydrogel are presented in Fig. 4. The PNIPAAm hydrogels exhibited porous, tubular-like structures with interconnected pores. However, the copolymerized P(NIPAAm-co-AAC-GA) hydrogel showed more porous network structures than pure PNIPAAm hydrogel. The pore dimension or mesh size of hydrogels decreased after the reaction with GA. The pore of P(NIPAAm-co-AAC-GA) hydrogel appeared to become smaller and more closed, which may result from the presence of complex molecules GA on the side chains in the copolymerized hydrogel.

Effect of temperature on the equilibrium swelling ratio

The copolymerized hydrogel will be used for investigation of cell attachment and detachment in which the cultured cells would attach to the hydrogel and form cell sheet at 37 °C and detach from the hydrogel by lowering the temperature. Therefore, the swelling behaviors of the hydrogel between 20 and 40 °C were investigated.

The effect of temperature on the equilibrium swelling ratio of hydrogels is shown in Fig. 5. It was seen that P(NIPAAm-co-AAC-GA) hydrogel was temperature responsive. The swelling ratio of P(NIPAAm-co-AAC-GA) hydrogel decreased with the increasing of temperature due to the enhanced hydrophobic interactions between hydrophobic groups. It was also found that the equilibrium

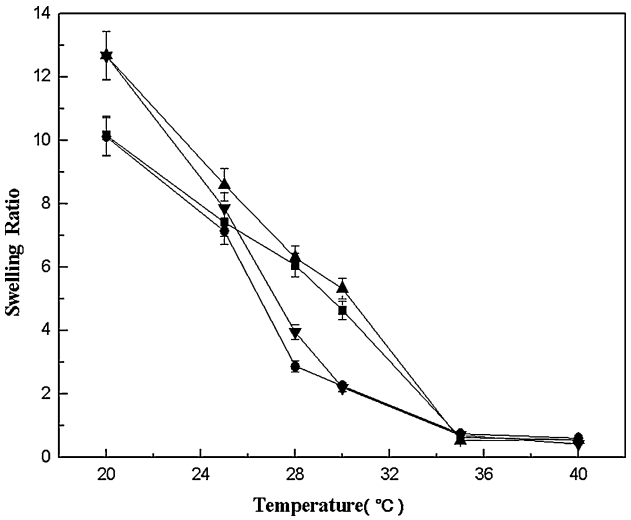


Fig. 5 Temperature dependence of the SR of PNIPAAm and P(NIPAAm-co-AAC-GA) hydrogel. P(NIPAAm-co-AAC-GA) in distilled water (*filled square*), P(NIPAAm-co-AAC-GA) in DMEM (*filled circle*), PNIPAAm in distilled water (*filled triangle*), PNIPAAm in DMEM (*filled inverted triangle*)

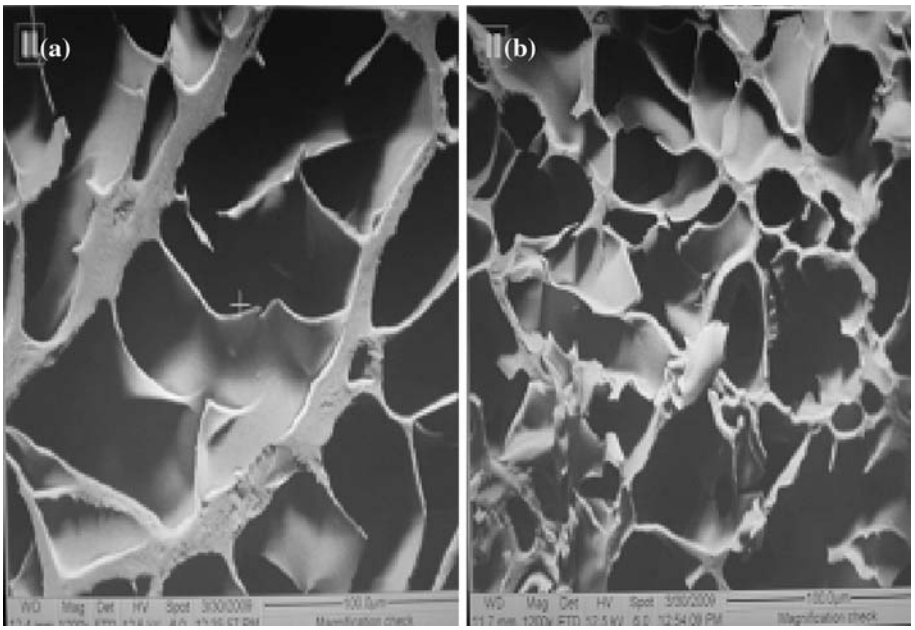


Fig. 4 Scanning electron micrographs of a cross section of **a** PNIPAAm hydrogel and **b** P(NIPAAm-co-AAC-GA) hydrogel

swelling ratio of copolymerized hydrogel was lower than that of PNIPAAm hydrogel resulting from the hydrophobic nature of GA. For example, the swelling ratios of P(NIPAAm-co-AAC-GA) hydrogel and PNIPAAm hydrogel in water are about 10.16 and 12.69 at 20 °C, respectively. In contrast to PNIPAAm hydrogel, the volume of P(NIPAAm-co-AAC-GA) hydrogel around LCST did not change as dramatically as PNIPAAm hydrogel. The reason was that PNIPAAm was soluble with a hydrated, extended chain conformation in aqueous solution below 32 °C and becomes insoluble due to the rapid, reversible chain dehydration and aggregation above this temperature. For P(NIPAAm-co-AAC-GA) hydrogel, the existence of hexahydric ring in GA strengthened the structural rigidity of the molecule which made it very difficult for the configuration to be changed. The steric hinderance resulting from the rigidity, hampered the transition of configuration and further weakened the transition between hydrophilic and hydrophobic states, which resulted in the slower volume change of P(NIPAAm-co-AAC-GA) hydrogel around LCST. Moreover, the temperature responsiveness of P(NIPAAm-co-AAC-GA) hydrogel mainly resulted from the PNIPAAm. There was relatively lower content of PNIPAAm in P(NIPAAm-co-AAC-GA) hydrogel than in PNIPAAm hydrogel, which also could not result in the drastic volume change of P(NIPAAm-co-AAC-GA) hydrogel at LCST.

Deswelling kinetics

The deswelling kinetics of hydrogels is presented in Fig. 6. As shown in this figure, P(NIPAAm-co-AAC-GA) and PNIPAAm hydrogels had similar response to the external temperature change in distilled water and DMEM. Abrupt shrinkage was observed in both hydrogels when the temperature was changed from 20 to 40 °C, and then they both reached the equilibrium state. However, the deswelling rate of P(NIPAAm-co-AAC-GA) hydrogel was faster than that of PNIPAAm hydrogel due to the hydrophobic nature of GA. For example, after 10 min at 40 °C, P(NIPAAm-co-AAC-GA) hydrogel in water lost about 50% water, while only 15% water was evacuated from PNIPAAm hydrogel in water. It took about 20 min and 60 min, respectively, for P(NIPAAm-co-AAC-GA) and PNIPAAm hydrogels to reach the equilibrium of deswelling.

Reswelling kinetics

The reswelling kinetics of hydrogel is displayed in Fig. 7. It can be found that the water uptake of two kinds of hydrogels showed rapidly swelling tendencies, and a rough equilibrium was reached after 1200 min, which suggested a rapid swelling and huge water absorbability for these hydrogels. However, during the reswelling process, the water uptake rate of P(NIPAAm-co-AAC-GA) hydrogel was slower than that of PNIPAAm hydrogel. For example, the water uptake of P(NIPAAm-co-AAC-GA) and PNIPAAm hydrogel was 6.6% and 116.9%, respectively, after 10 min. Even at equilibrium state (1500 min), the water uptake of P(NIPAAm-co-AAC-GA) and PNIPAAm hydrogels was 92.6% and 1733.8%, respectively. The water uptake of PNIPAAm hydrogel is about 20 times as much as that of P(NIPAAm-co-AAC-GA) hydrogels. Two main factors in these hydrogels may affect the water

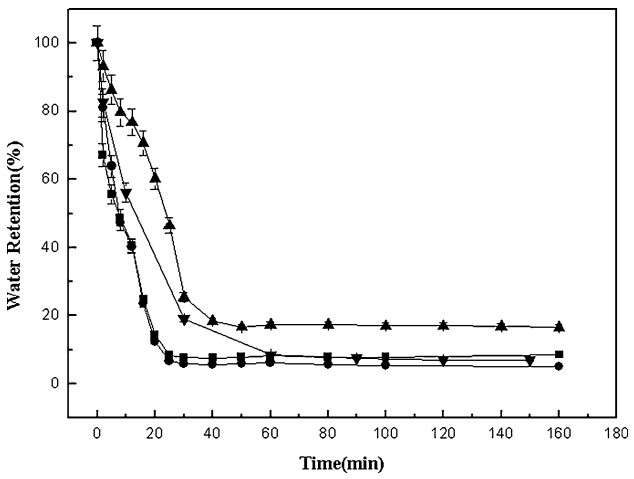


Fig. 6 Deswelling kinetics of P(NIPAAm-co-AAC-GA) hydrogel and PNIPAAm hydrogel. P(NIPAAm-co-AAC-GA) in distilled water (*filled square*), P(NIPAAm-co-AAC-GA) in DMEM (*filled circle*), PNIPAAm in distilled water (*filled triangle*), PNIPAAm in DMEM (*filled inverted triangle*)

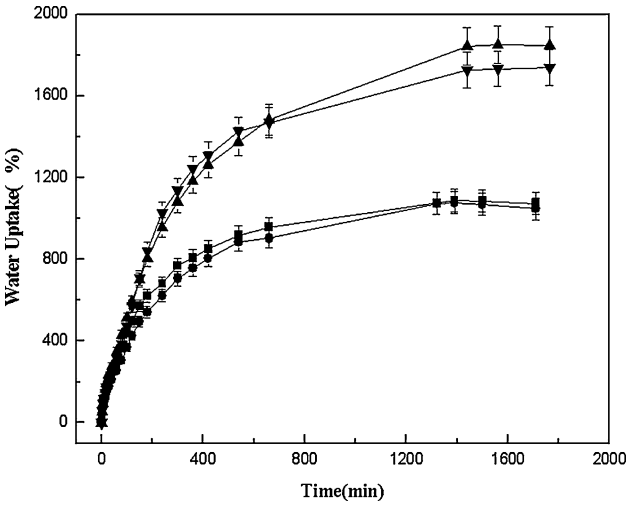


Fig. 7 Reswelling kinetics of P(NIPAAm-co-AAC-GA) hydrogel and PNIPAAm hydrogel. P(NIPAAm-co-AAC-GA) in distilled water (*filled square*), P(NIPAAm-co-AAC-GA) in DMEM (*filled circle*), PNIPAAm in distilled water (*filled triangle*), PNIPAAm in DMEM (*filled inverted triangle*)

uptake: the first one was that dry hydrogels first need to absorb water to overcome the interactions of polymer chains in order to relax the chain segment [5], whereas as discussed before, the existence of hexahydric ring in GA strengthened the structural rigidity of the molecule which hampered the movement of polymer chains; another factor was that the hydrophobic nature of GA in copolymerized hydrogel could prevent the entry of water.

Swelling reversibility of hydrogels

From the point of applications, the swelling reversibility of hydrogel was investigated in response to the temperature changes around the normal temperature (37 °C) of human beings, as shown in Fig. 8. P(NIPAAm-co-AAC-GA) hydrogel showed reversible thermo-responsive characteristics when temperature was changed periodically between 40 and 20 °C. In the reswelling process, the water retention recovered to the initial equilibrium state. This indicated that P(NIPAAm-co-AAC-GA) hydrogel had a suitable mechanical strength to undergo a reversible shrink process [30].

Step swelling behaviors were further investigated in temperature alternating between 40 and 20 °C. Figure 9 gave the water retention changes of hydrogels in predetermined period cycles. In the first cycle, temperature switched every 6 min because of the very fast deswelling rate. However, this interval was changed to 12 min in the following cycles. As shown in this figure, the swelling processes were proved to be repeatable with temperature changes though the decrease of magnitude for water retentions existed to some extent. The decrease of magnitude for water retentions in different temperature cycles can be explained by the viscoelastic characteristics of polymer chains which delayed the response of their conformations to temperature changes [5].

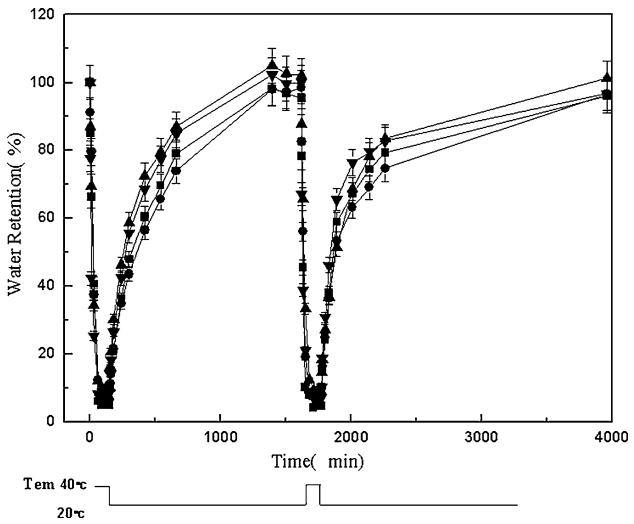


Fig. 8 Swelling reversibility of P(NIPAAm-co-AAC-GA) hydrogel and PNIPAAm hydrogel. P(NIPAAm-co-AAC-GA) in distilled water (*filled square*), P(NIPAAm-co-AAC-GA) in DMEM (*filled circle*), PNIPAAm in distilled water (*filled triangle*), PNIPAAm in DMEM (*filled inverted triangle*)

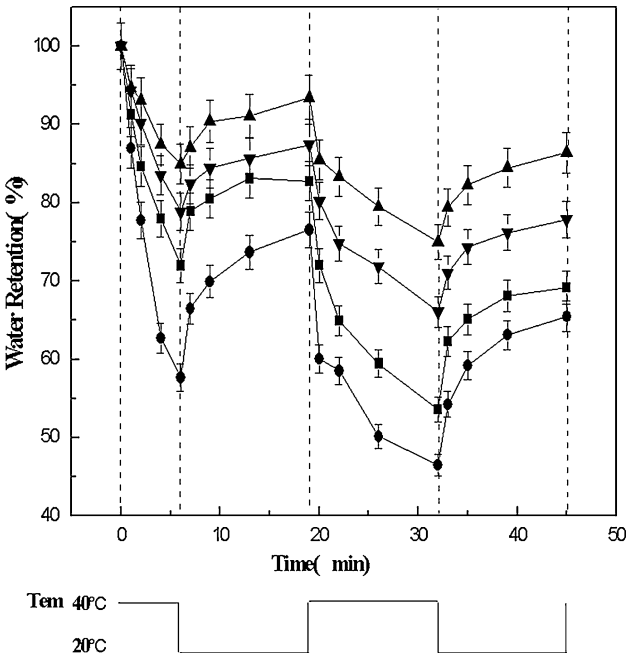


Fig. 9 Pulsatile temperature-dependent swelling behaviors of P(NIPAAm-co-AAC-GA) hydrogel and PNIPAAm hydrogel. P(NIPAAm-co-AAC-GA) in distilled water (*filled square*), P(NIPAAm-co-AAC-GA) in DMEM (*filled circle*), PNIPAAm in distilled water (*filled triangle*), PNIPAAm in DMEM (*filled inverted triangle*)

Based on these facts, P(NIPAAm-co-AAC-GA) hydrogel can be thought as reversible thermo-responsive materials. Thus, it can be expected as a kind of good material with improved properties for such application as tissue repairing and organ reconstruction fields.

Conclusions

In this study, novel temperature-responsive hydrogels containing hepatic affinity glycyrrhetinic acid (GA) were prepared successfully and their swelling behaviors were investigated. It was found that P(NIPAAm-co-AAC-GA) hydrogels were temperature responsive in both distilled water and cell culture medium. The swelling properties in both kinds of liquid were similar with only minor difference in their LCSTs (30.00 and 28.22 °C, respectively). The copolymerized hydrogel exhibited reversible thermo-responsive characteristics. All the results provided the basis for the subsequent experiments to investigate the behaviors of hepatic cell attachment and detachment. In addition, the incorporation of hepatic affinity glycyrrhetinic acid (GA)

into the P(NIPAAm-co-AAC-GA) hydrogel could especially be expected as good candidate materials for hepatic cell culture.

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