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Multi-responsive polyethylene-polyamine/gelatin hydrogel induced by non-covalent interactions

Zhidong Zhang, Yingxin Liu, Xin Chen and Zhengzhong Shao*

By simply introducing of gelatin aqueous solution, the polyethylene-polyamine (PPA)/gelatin hydrogel with multi-stimuli-responsive properties was obtained. It was demonstrated that the hydrogel was formed via non-covalent interactions, which were able to provide various stimuli-responsiveness such as stress, pH and thermal/photo-thermal to the hydrogel. Furthermore, the adhesion experiment revealed that the hydrogel was similar in adhesive strength but more convenient in preparation compared to other biomacromolecule based hydrogel adhesives, while it held more stimuli-responsiveness properties. Therefore, such a general strategy of the PPA based hydrogels preparation displayed its great potential to design smart materials for multi-functional applications.

Introduction

In recent years, smart hydrogels have attracted attention due to their multifunctional and responsive properties[1-4], which have been studied in various areas such as sensors[5-7], actuators[8, 9] and other smart equipment[10, 11]. These hydrogels were normally synthesized via chemical crosslinking to realize their multi-responsiveness. Compared to the chemical-crosslinked ones, physical-crosslinked hydrogels which were constructed through hydrogen bonding, ionic interaction, metal coordination, π–π stacking and hydrophobic interaction, etc.[12-14] have gained much more interest because of the low cost, mild and environmentally friendly preparations. However, a comprehensive and systematic physical approach to gain hydrogel with correlatively reversible and responsive abilities, such as pH, heat, photo-thermal and stress still remains challenging.

Among the smart properties of those physical-crosslinked hydrogels, stress responsive behavior has been one of the most important and favorable in adhesive and coating.[15-17] Varieties of materials and methods have been performed to obtain such smart hydrogels with industrial application potential. For example, Varghese et al achieved adhesive properties through introduction of pendant side chains from acryloyl-6-aminocaproic acid, which possess an optimal balance of hydrophilic and hydrophobic moieties.[18] Furthermore, Chen et al showed a design of hydrogen-bonding brush polymer supramolecular assemblies combining high modulus and

State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Laboratory of Advanced Materials, Fudan University, Shanghai, 200433, People's Republic of China.
E-mail: zzshao@fudan.edu.cn;
Tel: +86 21 6564 2866
Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

toughness with spontaneous healing capability, besides directly reacting with the molecules.[19] Nevertheless, combining stress responsive with other stimuli-responsive properties is rarely reported in hydrogel because ingenious crosslinking force is required. Therefore, inducing stimuli-responsive hydrogels capable of adhesive and coating are highly desirable for numerous applications.[20-22]

Polyethylene polyamine (PPA) is a liquid-like materials and a strong hydrogen bond supplier due to the numerous amine group ($–NH_2$) in its chemical structure, however, it is seldom reported as a practical hydrogel because of the restrictions of its non-responsive property as well as the weak interactions with other synthetic polymers.[23] On the other sides, a number of proteins have been widely applied in responsive hydrogels.[24, 25] For example, gelatin is able to form thermal induced hydrogel due to the non-covalent interactions between its molecular chains, but it is rare to be prepared as the smart material[26-28], mainly because of its poorly mechanical properties. In this work, we tried to produce PPA/gelatin composite hydrogel by straightforward mixing, and expected that the resultant hydrogel would present significant adhesive performances and multi-responsive abilities as the combination of PPA and gelatin may play the particular roles in terms of the network and properties of such hydrogel.

Experimental

Materials

PPA, gelatin and albumin from bovine serum (BSA) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). All the materials were used without further purification. Regenerated silk fibroin (RSF) from B.mori silkworm cocoons degummed by Na_2CO_3 and $NaHCO_3$ was acquired according to the literature.[29] Graphite powder (40 mm) was purchased from

Qingdao Henglide Graphite Co., Ltd. and oxidized to graphene oxide (GO) according to the previous reports.[30]

Preparation of PPA/gelatin (PPA/RSF and PPA/BSA) and PPA/gelatin/GO hydrogel

For PPA/gelatin hydrogels, the concentration of PPA was adjusted from 10 wt.% to 50 wt.%, and the concentration of gelatin in hydrogels ranged from 0.1 wt.% to 8 wt.% (Table.S1. For convenience, P50/G0.5 or P50/G6 represents the hydrogel mixed with 50 wt.% PPA and 0.5 wt.% or 6 wt.% gelatin, respectively, and so on).

For instance, P50/G6 hydrogel was prepared by slowly dropping 2.5 mL PPA into 2.5 mL 12 wt.% gelatin aqueous (the same process for RSF aqueous or BSA aqueous). The solution was gently and fully stirred in 7 mL centrifugal tube and put in 60 °C water base for 5 mins. Finally the solution was took out and cooled in room temperature for 30 mins and then transformed into hydrogel.

For P50/G6 with 0.1 wt.% GO hydrogel, it was prepared by mixing 1.25 mL 0.4 wt.% GO aqueous with 1.25 mL 12 wt.% gelatin aqueous. 2.5 mL PPA was slowly dropped into the gelatin/GO aqueous, and the solution was gently and fully stirred in 7 mL centrifugal tube and put in 60 °C water base for 5 mins. Finally the solution was took out and cooled in room temperature for 30 mins and then transformed into hydrogel.

DSC test

DSC was performed with P10/G6 hydrogel, P30/G6 hydrogel and P50/G6 hydrogel on a TA Q2000 instrument, with a heating rate of 2 °C per minute from 10 °C to 50 °C.

Rheology test

Rheology tests including time scanning, temperature scanning and frequency scanning experiments were performed on an Anton Paar MCR-301 rheometer. The PPA/gelatin, PPA/RSF and PPA/BSA hydrogels were tested on a plate−plate (PP-25) at 25 °C, 10 rad/s and under 0.05% amplitude strain in linear regime for their strength through time scanning. The PPA/gelatin hydrogels were tested on a plate−plate (PP-25) from 25 °C to 43 °C at 10 rad/s, and from 1 rad/s to 600 rad/s at 25 °C, respectively, under 0.05% amplitude strain in linear regime for their thermal and elastic behaviors through temperature and frequency scanning. All samples were stabilized for 10 mins before the measurement.

Gel-sol transition

For instance, P50/G6 hydrogel was put in 7 mL centrifugal tube, and the centrifugal tube was immersed in 45 °C water base for 3 mins. Then it was took out and put upside-down. If the hydrogel was not transformed into solution, the water base was then raised 1 °C and the experiment was repeated until the hydrogel transformed into solution. The gel-sol transition temperature was recorded and took average for 5 experiment results.

Adhesion experiment

For instance, about 0.2 grams weight P50/G6 hydrogel was covered around 2 cm × 2 cm area on glass, wood, PVC and Aluminum piece surfaces respectively and the pieces were bonded one with each other with 200 N load. All the samples were stabilized in room temperature for 1 day. Then tension test was performed on Instron 5565 to test the maximum tension stresses until the adhesive bond ruptured, averaged for at least 5 samples results. The Instron was equipped with a 200 N load cell and grip-apart rate was set as 0.33 mm/s. All samples were tested at 23 °C.
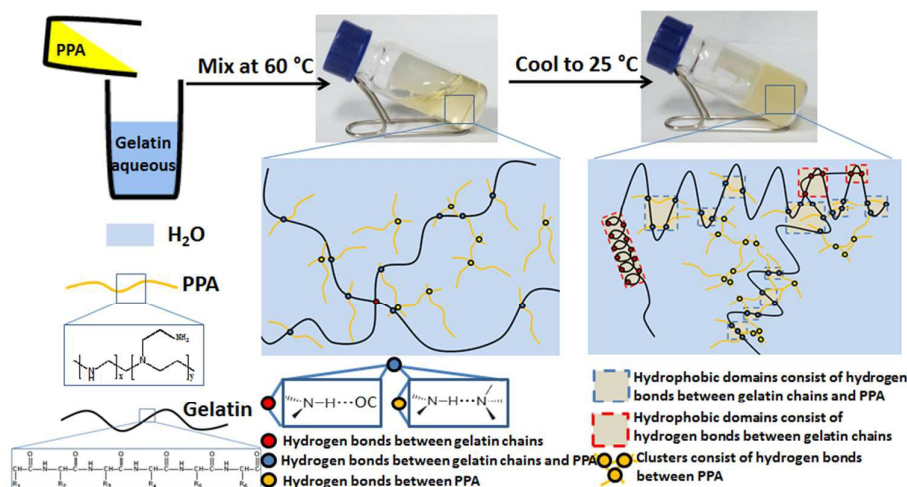
Fig.1 Illustration of preparation, hydrogen-bond and hydrophobic-domain categories in the PPA/gelatin hydrogel. When PPA and gelatin aqueous solution were mixed at 60 °C, there only existed few hydrogen bonds between gelatin chains and PPA (blue circles), gelatin chains (red circles) and PPA (yellow circles) in the solution. After cooling to 25 °C, the abundant hydrophobic domains consisting of hydrogen bonds between gelatin chains and PPA (blue squares) and gelatin chains (red squares) showed up due to the increasing formation of hydrogen bonds in the process of cooling.

Hydrogel drying experiment

About 0.2 grams weight P50/G6 hydrogel and P50/G2 hydrogel were covered around 2 cm × 2 cm area on glass piece surfaces respectively. The weights of the hydrogels were recorded as M_0. Then the samples were put into dryer and stabilized in room temperature. During stabilization, the weights of the hydrogels were recorded as M_t for 1, 2, 3, 4, 5 and 7 days, respectively. The experiment results were averaged for at least 6 samples.

Results and discussion

PPA/gelatin hydrogel was acquired by simply mixing PPA into gelatin aqueous solution and gently stirring at 60 °C for 5 mins, followed by cooling in room temperature. The prepared hydrogel was gradually dissolved by strong hydrogen bonding breaker such as urea and swollen by water (Fig.S1). It was speculated that various categories of hydrophobic domains and clusters consisting of different hydrogen bonds formed in the hydrogel and all of them contributed to the physically crosslinked network. As illustrated in Fig.1, in the process of PPA/gelatin solution cooling to 25 °C, numerous amine groups of PPA molecules mainly formed hydrogen bonds with carboxyl groups of gelatin, thus created the major hydrophobic domains as the dominant cross-linkers. Certainly, amine groups and carboxyl groups of gelatin and amine groups of PPA molecules themselves could also form some "private" hydrophobic domains and clusters, respectively.

As known to all, energy such as absorbing heat is one of the driving forces to dissociate hydrogen bonds.[31] In the case of PPA/gelatin hydrogel, the hydrophobic domains and clusters organized by three kinds of hydrogen bond may be monitored by DSC measurement, because the dissociation temperature of various hydrogen bonds with distinctive activation energy differs from each other.[32] It could be seen that there were three endothermic peaks displayed in DSC curve of each PPA/gelatin hydrogels (Fig.2). After comparing to those of control samples,
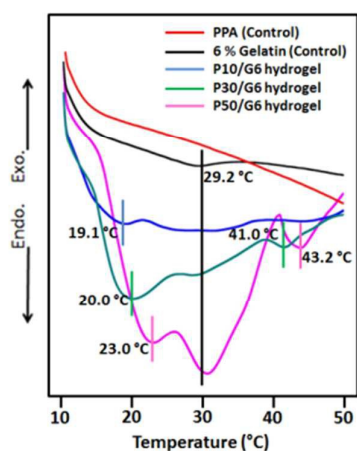
Fig.2 DSC profiles of PPA, gelatin hydrogel and PPA/gelatin hydrogels with different PPA concentrations.
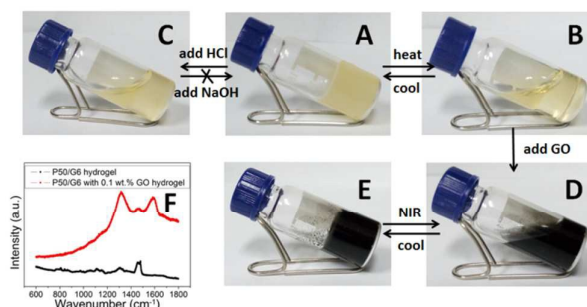
Fig.3 Responsive characters of PPA/gelatin and PPA/gelatin/GO hydrogels. (A) PPA/gelatin hydrogel at room temperature; (B) PPA/gelatin solution at 60 °C, noting the reversible gel-sol transition through heating and cooling process; (C) PPA/gelatin suspension induced by acidic agent, noting the irreversible gel-sol transition; (D) PPA/gelatin/GO aqueous solution at 60 °C; (E) PPA/gelatin/GO hydrogel at room temperature, noting the reversible sol-gel transition of PPA/gelatin/GO driven by exposing to NIR for 5 min and cooling; (F) Raman spectra of PPA/gelatin and PPA/gelatin/GO hydrogel.

we summarized that the peaks around 20 °C, 30 °C and 42 °C possibly corresponded to the breakings of hydrogen bond formed by PPA/PPA, gelatin/gelatin and PPA/gelatin, respectively. When the concentration PPA increased from 10 wt.% to 50 wt.%, the endothermic peak of hydrogen bond formed by gelatin itself (around 30 °C, from Fig.2) hardly shifted, suggesting the involvement of PPA might not affect such hydrogen bond. However, with the increasing of PPA, the endothermic peaks of PPA/PPA hydrogen bond and PPA/gelatin hydrogen bond significantly moved to higher temperatures (PPA/PPA, from 19 °C to 23 °C and PPA/gelatin, from 41 °C to 43 °C, respectively), indicating that the addition of PPA created more hydrogen bonds in the hydrophobic domains and clusters which consumed more energy for dissociation. Additionally, rheological modules of the gelatin hydrogel and PPA/gelatin hydrogel in the different temperatures were employed to confirm that the hydrogen bond formed by PPA and gelatin were more stable than that between gelatin chain itself, as the G' of the gelatin hydrogel and its counterpart dramatically dropped in the temperature of 28 °C and 38 °C, respectively (Fig.S2). The results from DSC measurement and rheological modules convinced that the dominant cross-linkers were provided by PPA/gelatin hydrogen bond.

PPA/gelatin hydrogel had a reversible gel-sol transition with the temperature (Fig.3A and 3B), due to the physically crosslinked network which constructed through hydrophobic areas consisting of thermal-sensitive hydrogen bonds. The anastrophy method was carried out to test the gel-sol transition temperature of PPA/gelatin hydrogel with different compositions. Fig.4A showed that the gel-sol transition temperature of the hydrogel varied from 34 °C to 48 °C when the concentration of gelatin increased from 0.25 wt.% to 6

wt.%. Besides providing the information that the gel-sol transition temperature of PPA/gelatin hydrogel could be finely tuned by altering the compositions, the result further confirmed that the physical crosslinked network in the PPA/gelatin hydrogel differs from that of in gelatin one because the gel-sol transition temperatures of pure gelatin hydrogel (without PPA) are usually around 32 °C. Moreover, the PPA/gelatin hydrogel displayed the irreversible collapse to be an opaque suspension after adding acid (Fig.3C). This phenomenon was reasonably due to the protonation of amine groups on PPA branches disassociated the hydrogen bonds between PPA and gelatin, and the hydrophobic force drove the aggregation of PPA itself. Slight PPA aggregations might still exist in the solution when NaOH was added, and they fail to form adequate crosslinking points to afford the establishment of hydrogel network (Fig.S3).

Graphene oxide (GO) is well known to its excellent photo-thermal effect.[33] To provide the photo-thermal response of the PPA/gelatin hydrogel, 0.1 wt.% GO was introduced into the solution of PPA and gelatin in relatively high temperatures (Fig.3D). After cooling down, the mixture turned to the gel state as PPA/gelatin did (Fig.3E). Nevertheless, the mixture of PPA, gelatin and GO could be switched between hydrogel and solution by cooling and raying of near infrared (NIR). Obviously, this was because of the uniformly dispersion of GO in the mixture, as Raman spectrum of the hydrogels showed that the two dominant peaks of 1346 and 1592 cm^{-1} (Fig.3F). The two peaks were assigned to D and G band of graphene, which were attributed to the disordered structure and the vibration of sp^2-bonded carbon atoms, respectively.[34]

Indeed, such hydrophobic domains as physical cross-linkers described in Fig.1 could be generally provided by various proteins. A few of other proteins, for example, regenerated silk fibroin from silk fiber (RSF) or albumin from bovine serum (BSA) is able to form the hydrogels with PPA in the contain conditions, as shown in Fig.S1.
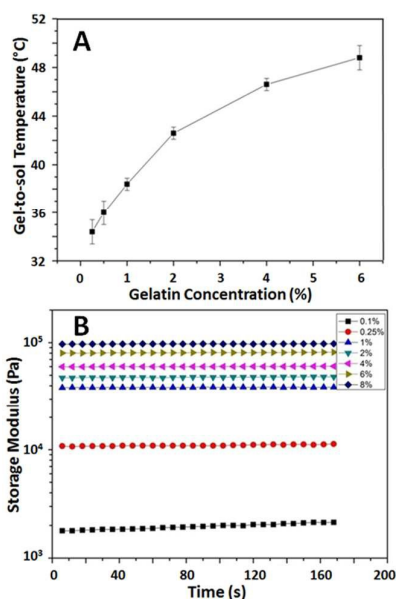
Fig.4 (A) Gel-sol transition temperature and (B) rheological storage modulus of PPA/gelatin hydrogel with different gelatin concentrations. Mass fraction of PPA is 50%, and the hydrogel is only formed in the case of gelatin involving.
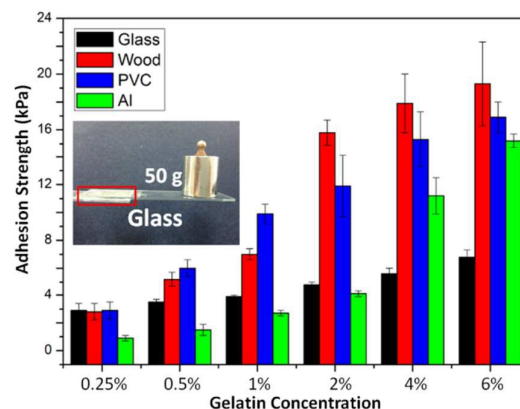
Fig.5 Adhesive strengths of the PPA/gelatin hydrogels with different compositions to various materials. Before the testing, the samples were dried in room temperature for 1 day (in such a case, the evaporation of the water in hydrogel was around 50%, referring to Fig.S8).

It should be noted that the mechanical properties of hydrogel are important to afford daily application and ideally should be adjusted for different demands. As mentioned above, PPA/gelatin hydrogel were mainly formed through hydrophobic domains produced by the package of hydrogen-bonds between PPA and gelatin. So it was possible to realize the control of the hydrogel modulus through altering the proportion of PPA and gelatin, which may correspond to the number of hydrogen-bond as well as crosslinking point. As shown in Fig.4B, the G' of the hydrogel was greatly increased after the introduction of a little amount of gelatin, and varied from 2×10^3 Pa to 10^5 Pa when the concentration of gelatin increased from 0.1 wt.% to 8 wt.%. Consistently, the rheological modulus of the hydrogels with the certain amount of gelatin was enlarged by the increasing of PPA contents (Fig.S4). Certainly, the modulus of the hydrogel formed by PPA and RSF or BSA also could be adjusted through altering the concentration and the molecular weight of the protein (Fig.S5 and Table.S2.).

Indeed, the PPA/gelatin hydrogel could be applied as an adhesive for various materials, because it not only performed the significant mechanical properties, but also was organized by polar molecules and hydrophobic domain via strong internal interaction. In the case of adhesive testing, glass, wood, polyvinyl chloride (PVC) and Aluminum, which are represented to inorganic material, natural polymer, synthetic polymer and metal, respectively, were employed as the substrates. The results showed the universal adhesion strength of the hydrogel relied on the concentration of gelatin (Fig.5). Moreover, it was speculated that the adhesion strength differing from these materials mainly resulted from different physical interactions through hydrogen bonding on material/hydrogel interface, and higher adhesion strength of wood and PVC was

probably owing to the polar functional groups in their molecular structures, which might develop stronger physical interactions with amine groups of PPA on material/hydrogel interface. In fact, such hydrogel adhesive even could work on polytetrafluoroethylene (PTFE)-one of the most hydrophobic polymers (Fig.S6A). Interestingly, P50/G6 hydrogel also revealed its self-healing property in the room temperature (Fig.S6B and S6C). The most likely explanation was that those physical cross-linkers of the network in the hydrogel were based on the dynamic hydrogen bonds. Furthermore, the hydrogel adhesive could be quite stable in water but be destroyed in acidic aqueous solution due to its pH-responsive property (Fig.S7). Comparing to other hydrogel adhesives prepared by biomacromolecules[35], P50/G6 hydrogel displayed similar in adhesive strength while held more convenient producing process and stimuli-responsiveness properties.

Conclusions

We successfully prepared PPA/gelatin hydrogel through a convenient blending method. We explored the non-covalent interactions in this hydrogel and confirmed the hydrophobic domains as physical cross-linkers of PPA/PPA, gelatin/gelatin, and PPA/gelatin by DSC analysis. Indeed, such non-covalent interaction could be induced by various proteins such as regenerated silk fibroin or albumin to produce the PPA/protein hydrogel. It was found that the storage modulus of the hydrogel might be adjusted by controlling the concentration or the molecular weight of those involved proteins. Practically, the PPA/gelatin hydrogel revealed thermal-responsive, pH-responsive and stress-responsive properties and proved to be possible to be used as multi-responsive adhesive. Moreover, with the introduction of GO, PPA/gelatin/GO hydrogel could be provided with photo-thermal property and this implied more opportunities to explore the application due to its convenience and low cost.

Acknowledgements

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