

Supplementary Information

Injectable and Fast Self-Healing Protein Hydrogel

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1. Materials and instruments

Materials

Bovine serum albumin (BSA) was purchased from Bioengineering Inc. (Shanghai, China); Tris(2-carboxyethyl)phosphine (TCEP, 98%) was purchased from Beijing Bailingwei Technology Inc; dithiothreitol (DTT) was purchased from BioFroxx; Glutathione was purchased from Angie Chemical; 50% glutaraldehyde was purchased from Tianjin Huadong Reagent Factory.

Instruments

Heto lyolab 3000 Lyophilizer; Autoflex speed TOF/TOF Matrix-assisted Flight Mass Spectrometer; JEOL JSM-6700F Scanning Electron Microscope; Bruker Biologic PMS450 Circular Dichroism; MCR 302 Rheometer; Instron-5940 Tensile Instrument; OLYMPUS DP72 Optical Microscope; VERTEX 80V Fourier Transform Infrared Spectrometer (Brucker).

2. Infrared spectroscopy test

The prepared sample is ground to a fine powder with a mortar, and then the sample (1-2mg) is mixed with KBr powder (70mg). Hold in a tablet press for 5 minutes under a pressure of 10 tons, and press it into a round sheet to be tested. Finally, infrared spectroscopy was performed by a VERTEX 80V Fourier transform infrared spectrometer (Brucker). As we designed, there is no sulfhydryl group peak in the infrared spectrum of BSA. However, the sulfhydryl groups peak appeared in the infrared spectrum of the hydrogel sample, and the stretching vibration of -SH was observed at 2590-2540 cm^{-1} as shown in **Fig. S1**. This indicates that the TCEP reducing agent disconnects the disulfide bond in the BSA, resulting in a large amount of free sulfhydryl groups.

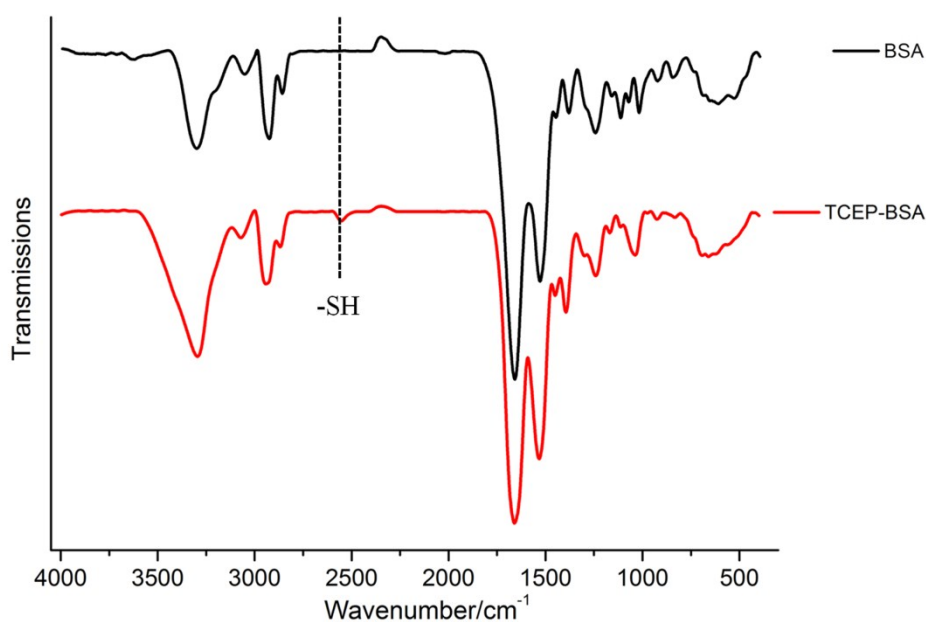


Fig. S1 The Infrared spectrum of BSA hydrogels.

3. Optical microscope test

The optical microscopy test for scratch repair more directly demonstrates the self-healing process of hydrogels. We have been treated with two different color hydrogel tablets and observed by OLYMPUS DP72 optical microscope. First, we prepared a protein hydrogel sheet on a glass slide, as shown in **Fig. S2(a)**, and used blade to gently draw a scratch on the surface of the protein hydrogel. Then a small amount of H_2O_2 was added in situ at the scratch. About 2 minutes later, under the optical microscope, it can be observed that the deep scratches of about 10um are repaired into very fine marks, as shown in **Fig. S2(b)**. In order to better observe the scratch repair effect, we dyed the BSA protein hydrogel into red with Rhodamine B, as shown in **Fig. S2(c)**. Then in the same way, a scratch was formed on the surface of the hydrogel and used H_2O_2 to promote scratch repair. Under 2 minutes, it can be more clearly observed under the optical microscope that the deep scratches of about 10um are repaired into very fine marks, as shown in **Fig. S2(d)**. These results indicate that the stimulatory response of H_2O_2 accelerates the repair of BSA protein hydrogel scratches and is a good protein hydrogel self-healing material that can be used for applications.

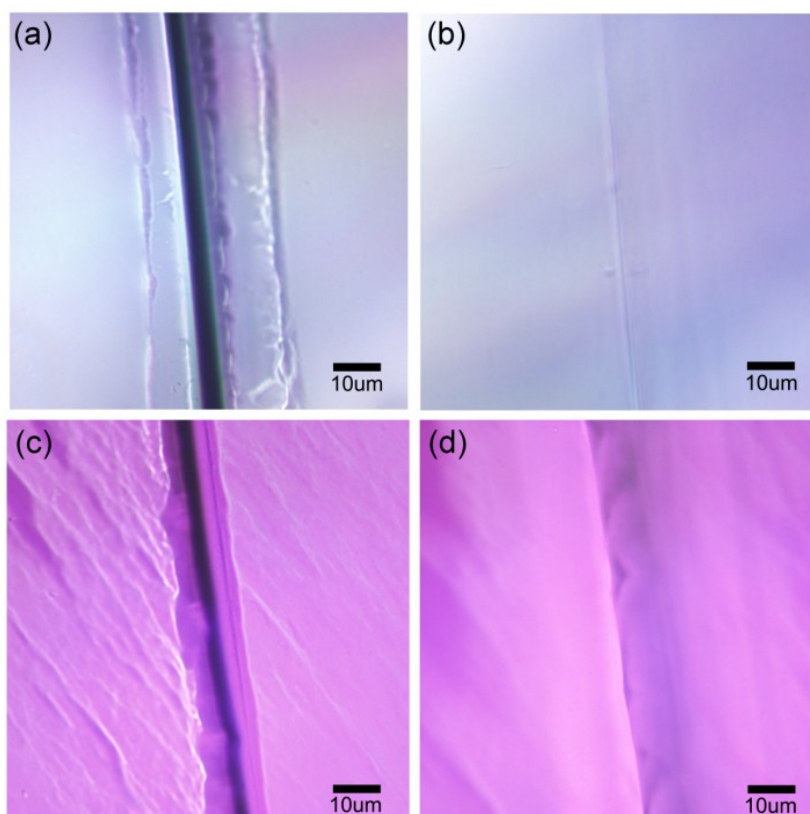


Fig. S2 The scratch repair photos of the hydrogel. (a) The protein hydrogel with a scratch. (b) The scratch repair photo of the protein hydrogel after 2 minutes. (c) The scratched hydrogel was stained with Rhodamine B. (d) The scratch repair photo of the hydrogel with Rhodamine B.

4. Circular dichroism test (CD)

We performed CD spectroscopy in a 0.1 cm quartz cell using a MOS-450/AF-CD spectropolarimeter (Bio-Logic, France) equipped with a thermostatic cell holder. The total amount of sample in the CD measurement was 400 μ L. For CD studies, spectra were acquired at 25 °C using 1 nm interval, 1 nm bandwidth, and a scan rate of 60 nm min⁻¹. Baseline correction before conversion to average residue ellipticity. The chemical denaturation curve was measured by collecting the spectra by an average of 3 repeated scans with a protein having a concentration of 3 μ M, and monitoring the ellipticity of BSA in the presence of different reducing agents at different times.

As shown in **Fig. S3**, the CD test was performed after adding the three reducing agents respectively for 1 h and 48 h. The results show that the CD test curve of the reduced BSA is almost identical to the characteristic curve corresponding to the CD curve of the BSA itself. The addition of the reducing agent simply breaks the disulfide bond in the BSA protein structure without altering its secondary structure. This also excludes the possibility of hydrogel formation due to protein denaturation. In addition, the hydrogel formed by the reduction of BSA protein also maintains the properties of the protein itself, which also provides a feasible way for the crosslinking of other functional proteins.

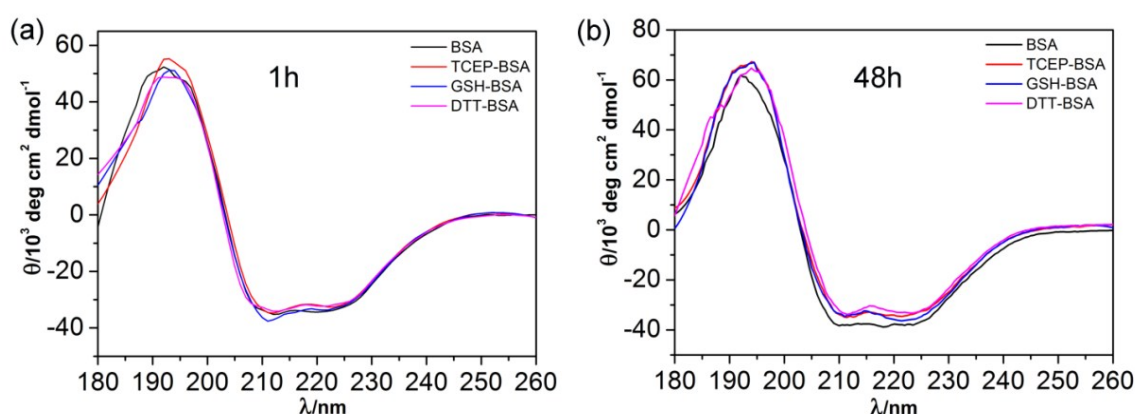


Fig. S3 (a) The CD measurements of the protein-based hydrogels with different reducing agent after 1 hour. (b) The CD measurements of the protein-based hydrogels with different reducing agent after 48 hours.

5. Injectable video

In order to more intuitively observe the injectability of the hydrogel, we performed a pinhole syringe extrusion experiment. We put the transparent hydrogel into a pinhole syringe and squeeze it out slowly. As can be seen from the video, the reduction-induced hydrogel can be continuously extruded from the pinhole as shown in video S5-1. In addition, in order to better observe the combination of shear thinning and self-repair, we add two different colors of the hydrogel to the pinhole syringe at the same time. One of them is a transparent hydrogel, and the other is a hydrogel dyed

with Rhodamine B. As shown in video S5-2, these two color hydrogels can be continuously extruded from a pinhole syringe.. This shows that the two color hydrogels also complete the self-repair process during extrusion. We combined shear thinning with self-healing properties to obtain a more complete hydrogel material.

6. Swelling and stability measurement

We tested the stability of the hydrogel as shown in **Fig. S4**. Firstly, two identical hydrogels were prepared. Both hydrogels were dyed red with Rhodamine B. Then one of the hydrogels was placed in a closed air and the other was soaked in water (pH=7) as shown in **Fig. S4(a)**. After observation at different times, we found that the hydrogel sealed in the air is stable and remains essentially unchanged, while another hydrogel that is in the water slowly degrades. The immersion in water hydrogel for 1 hour, it can be observed that Rhodamine B molecules diffuse into the water as shown in **Fig. S4(b)**. The hydrogel slowly expands with time, until about 15 hours, the hydrogel begins to decompose, and finally about 21 hours completely degraded and dissolved in water as shown in **Fig. S4(c-i)**. It also proves that the hydrogel has good degradability and completely degrades in water in about 21 hours.

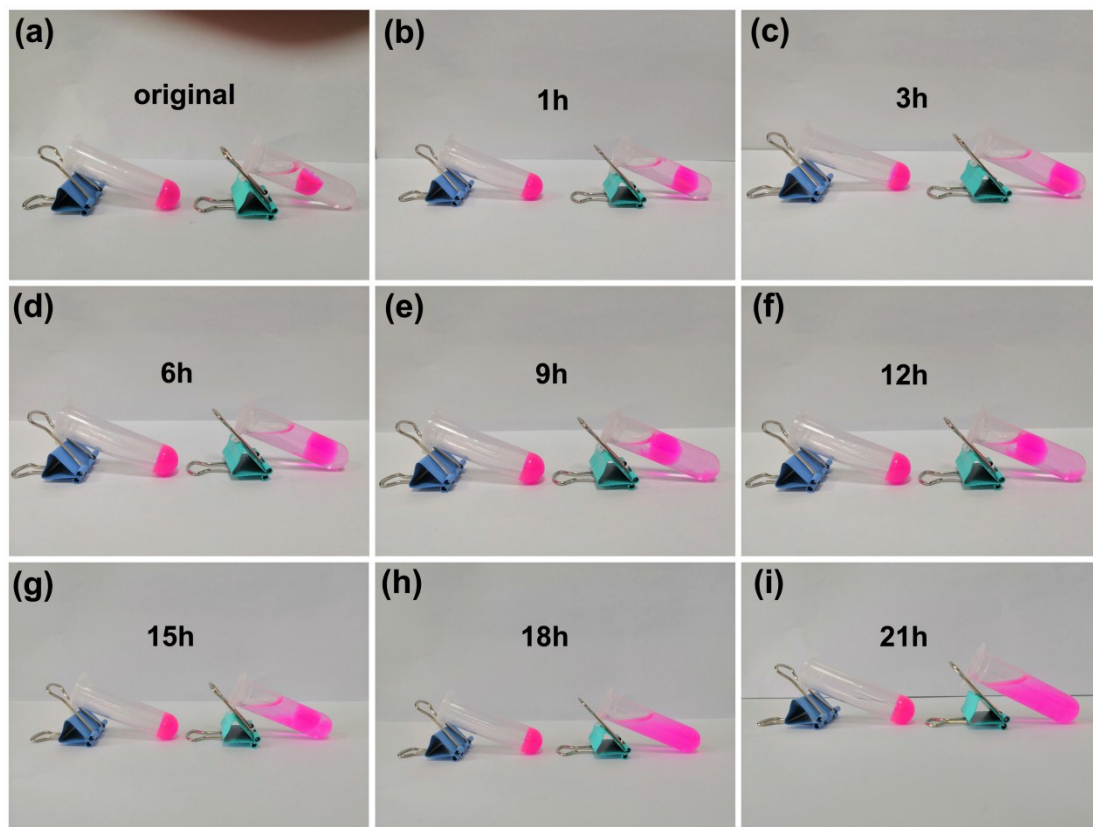


Fig. S4 The injectable protein hydrogel degradation experiment: The injectable protein hydrogel degradation experiment: (a)-(i) are the photos of the hydrogel degraded in water for different times.

For swelling measurements, ring-shaped hydrogels were weighted immediately after being taken out of mould, and the weight was recorded as M_0 . Hydrogels were then soaked in deionized water (pH=7) at the room temperature. After certain time, the hydrogel rings were taken out of deionized water, blotted onto tissue paper to remove excess deionized water and weighted as M_t . The swelling ratio was calculated according to the formula: Swelling ratio (%) = $[(M_t - M_0) / M_0] \times 100\%$.

As shown in **Fig. S5**, as the soaking time increases, the weight of the two protein hydrogels also gradually increases (**Fig. S5(a)**), and the swelling ratio also gradually increases (**Fig. S5(b)**). However, the difference is that the thiol/disulfide exchange protein hydrogel has a much higher swelling rate than the glutaraldehyde crosslinked protein hydrogel. After 15 hours, the thiol/disulfide exchange protein hydrogel

swelling rate reached a maximum of about 170%, after which the swelling rate of the hydrogel rapidly decreased. This is because the thiol/disulfide exchanged protein hydrogel is very stable before soaking for 15 hours, and begins to degrade after 15 hours, and a part of the hydrogel is decomposed and dissolved in water. In contrast, the swelling rate of glutaraldehyde crosslinked protein hydrogels has been increasing. In summary, the injectable and fast self-healing protein hydrogel has a certain stability and good degradation performance through swelling experiments.

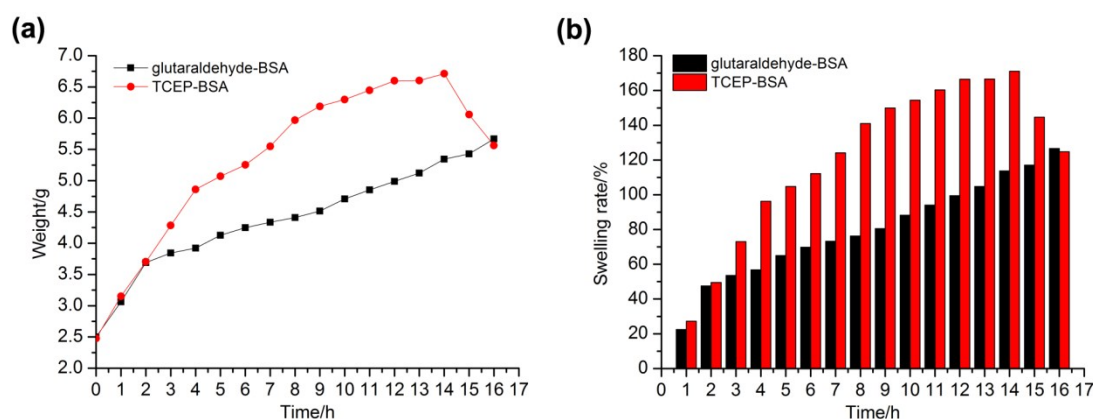


Fig. S5 Swelling measurements of the thiol/disulfide exchanged protein hydrogel and glutaraldehyde crosslinked protein hydrogels: (a) Soaking time and weight curve of these two hydrogels. (b) Swelling rate curve of these two hydrogels.

7. Viscosity test

To demonstrate that the hydrogel has a shear thinning behavior, we tested the viscosity of the hydrogel as shown in **Fig. S6**. As the shear rate applied to the hydrogel gradually increases, the viscosity of the hydrogel gradually decreases. Conversely, as the shear rate applied to the hydrogel gradually decreases, the viscosity of the hydrogel gradually returns to its original value. The results demonstrate that the hydrogel has the effect of shear thinning.

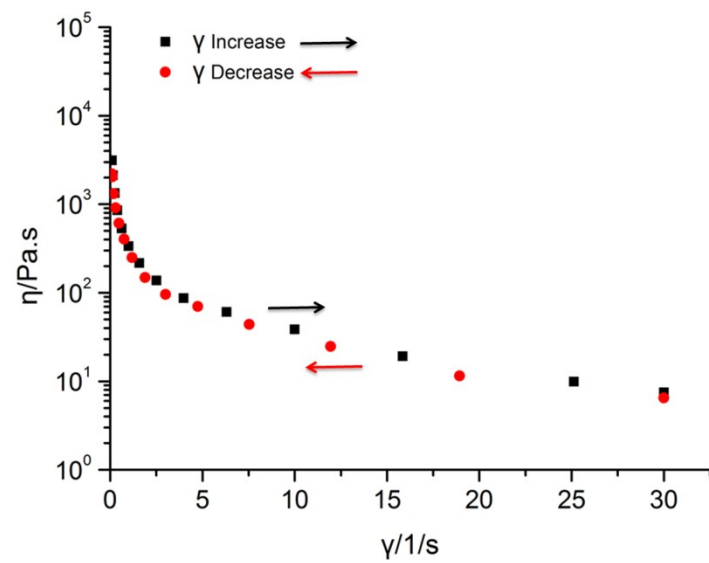


Fig. S6 Viscosity test of the thiol/disulfide exchanged protein hydrogel