Supplementary Information (ESI)

An injectable serotonin-modified chondroitin sulfate hydrogel for bio-inspired hemostatic adhesives with high wound healing capability

Xingxia Zhang,[a,b] Zhifang Ma, [a] Yue Ke,[a] Yu Xia,[a] Xiaodong Xu,*[b] Jingchuan Liu,[a] Yumei Gong,[c] Qiang Shi,*[a] and Jinghua Yin[a]

a State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

b Key Laboratory of Superlight Materials and Surface Technology, Ministry of Education, College of Materials Science and Chemical Engineering, Harbin Engineering University, Harbin 150001, China.

c School of Textile and Material Engineering, Dalian Polytechnic University, Dalian 116034, P. R. China

Content:

1. FTIR and NMR spectra of serotonin-modified chondroitin sulfate hydrogel (CSS)

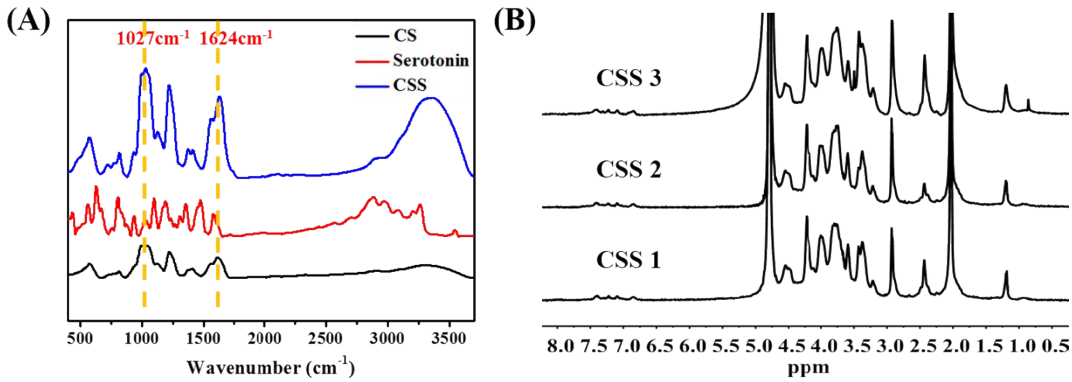

Figure S1. (A) FT-IR spectra of CS (black), Serotonin (red) and CSS (blue). (B) ^{1}H NMR (500 MHz, D_2O) spectra of CSS1, CSS2 and CSS3.

2. Rheological measurement

Rheological properties of the hydrogels were characterized by a model MCR 702 rheometer. The pre-gel solution containing CSS, H_2O_2 and HRP were in situ injected into parallel plate (plate diameter = 25 mm, gap = 0.5 mm) with a double-tube syringe, and silicone oil was dripped around the mixture to prevent evaporation of water from the hydrogel. The storage modulus (G′) and loss modulus (G") of the hydrogel were measured in a frequency sweep mode, and the linear viscoelastic zone of the hydrogel was determined by observing the changes of the two. The strain value (1 %) in the linear viscoelastic region of hydrogel was selected as the strain in a time sweep mode, and the rheometer test frequency was 1 Hz.

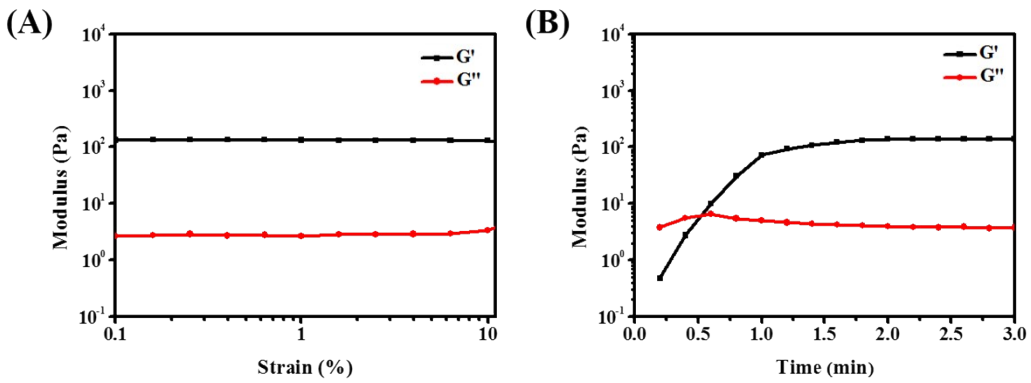

Figure S2. (A) Storage modulus (G′) and loss modulus (G") of CSS hydrogel crosslinked using 18 U/ml HRP and 4 mM H_2O_2 were measured at strains ranging from 0.1 to 10 % in strain sweep mode using a rheometer. (B) Gelation kinetics of CSS hydrogel crosslinked using 18 U/ml HRP and 4 mM H_2O_2 were analyzed by a time dependent rheological measurement.

3. Cell viability of CSS hydrogel

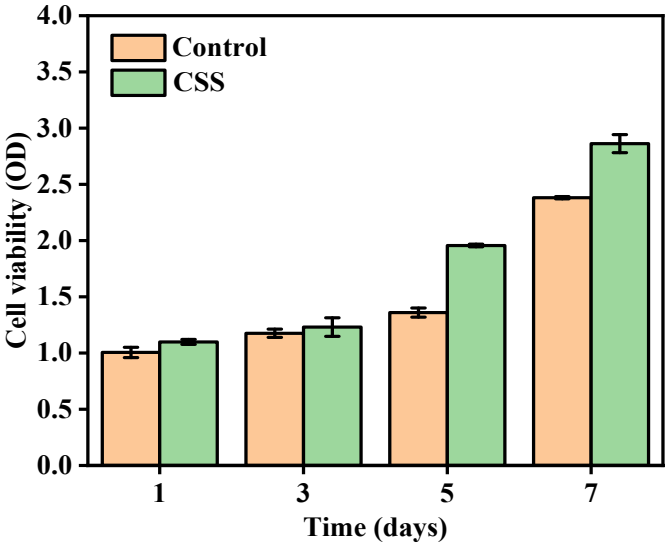

Figure S3. Cell viability of L929 murine fibroblasts after incubation with hydrogel extracts for 1, 3, 5, 7 days.

4. Living cell quantity

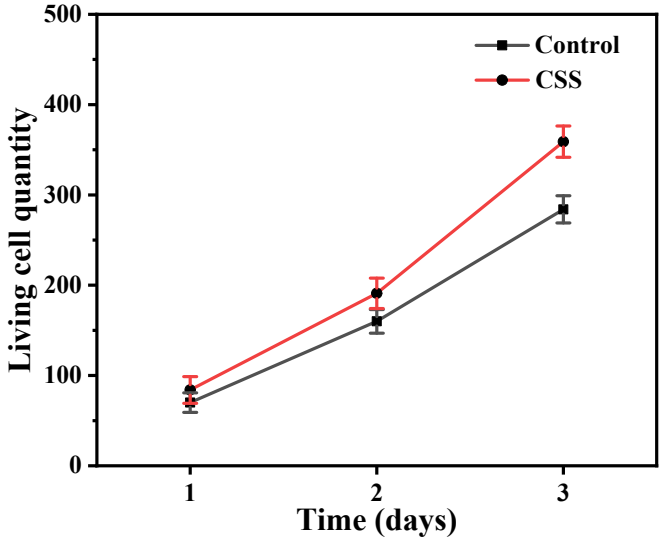

Figure S4. Living cell quantity after incubation with the hydrogel extracts for 1, 2, 3 days.

5. Supplemented mouse liver hemostasis experiments

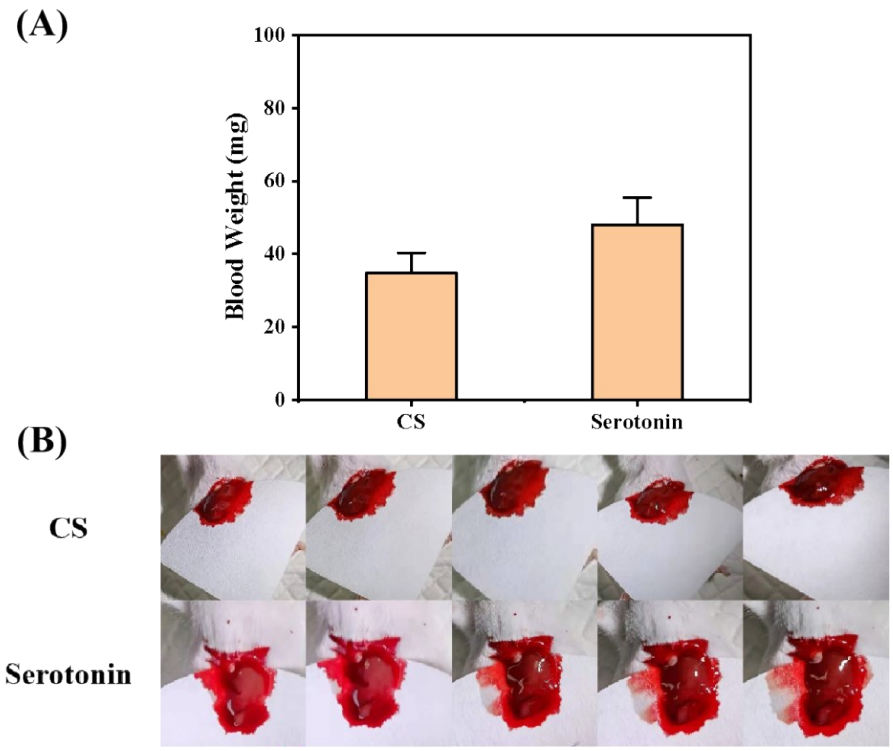

Figure S5. (A) Total blood loss from the damaged livers at 120 s treated with CS only and serotonin only. (B) Images of the bleeding mouse liver treated with CS only and serotonin only every 30 s for 2 min.

Mouse liver hemostasis experiments were performed with only serotonin and only CS versus CSS hydrogels. As shown in the Figure S5, compared with the serotonin group, CS group had a certain hemostatic effect, because it could form a sticky substance, which was conducive to preventing the bleeding of the wound. However, compared with the CSS hydrogel group, the effect of CS group was poor. Serotonin and CS as the main components of hydrogels play an important role, but only serotonin or CS cannot be prepared into hydrogels. Injectable hydrogel can accurately and quickly form gel in the wound site. First, it blocks and adheres to the bleeding site. Then, with the help of porous three-dimensional structure, it aggregates blood cells and releases major components to activate blood cells, thus achieving good hemostasis effect. Not only the main components of hydrogels, but also the internal structure and adhesion of hydrogels play an important role in the liver

bleeding experiment of mice. Therefore, CSS hydrogels have a higher hemostasis than wound bleeding treated with serotonin and CS only.

6. Histological analysis of the mice liver

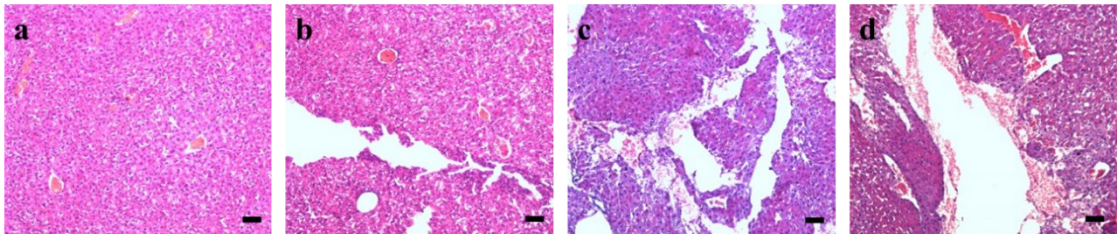

Figure S6. Histological analysis (H&E staining) of the mice liver. a. normal liver, b. the damaged liver without treatment, c. the damaged liver treated with commercial gum for 3 days after the hemostasis test. d. the damaged liver treated with CSS hydrogels for 3 days after the hemostasis test. (Scale bars =200 μm)

7. Preparation of aloe vera powder (AVP) doped CSS hydrogel

A series of CSS-AVP polymer solution was prepared by dissolving the CSS polymer and aloe vera powder in phosphate buffer solution (PBS: 0.01 M, PH 7.4) in a ratio of 5:1 (mass ratio), which was then divided into two equal parts. The polymer solution was mixed with equal volume H_2O_2 and HRP respectively, and then gently mix the two ingredients with a double-tube syringe to form an in-situ hydrogel. The hydrogel was freeze-dried in a vacuum freeze-dryer, and then the chemical structure of the raw material and the product was characterized by a Fourier Transform Infra-red spectroscopy (FT-IR).

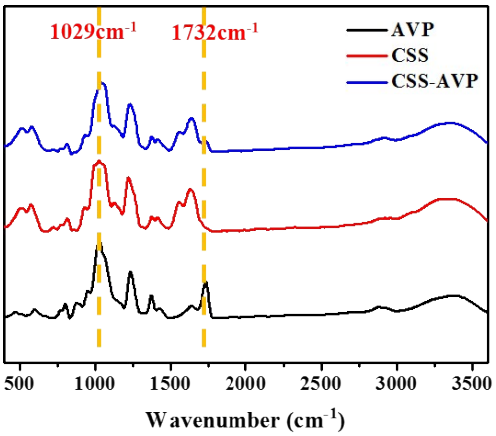

Figure S7. FT-IR spectra of AVP (blue), CSS (red) and CSS-AVP (black).