

Supplementary material

A Dual Network Composite Hydrogel with Robust Antibacterial and Antifouling Capabilities for Efficient Wound Healing

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Staining procedure

1. HE staining procedure

- (1) Paraffin tissue sections with a thickness of 3 μ m were lined on adhesive coated slides and baked in an oven (65°C) for 30 min;
- (2) Tissue sections were first deparaffinized by xylene for 10 min \times 2 times, then 95% ethanol for 5 min \times 2 times, 80% ethanol for 2 min, and rinsed with tap water for 2 min;
- (3) Transferred into hematoxylin staining solution for 8 min, slightly washed with running water for 3 s, put into 1% hydrochloric acid ethanol for 5 s, rinsed with running water for 20 min;
- (4) Put into 0.5% eosin staining solution for 5 min, slightly wash with running water for 5 s;
- (5) Dehydrate by gradient alcohol and bake dry, seal the slides with neutral gum and coverslip, and observe under microscope.

2. IHC staining steps

- (1) Paraffin tissue microarrays with a thickness of 4 μ m were lined on glued slides and baked in an oven (65 °C) overnight;
- (2) Tissue sections were first deparaffinized by xylene for 3 min \times 3 times, then dehydrated by 100%, 80% and 60% alcohol for 2 min each, and rinsed by tap water for 2 min;
- (3) Citrate buffer autoclave antigen repair: sections were immersed in 0.01M pH=6.0 citrate buffer that had been boiled, covered with an autoclave lid and a pressure valve, heated to a jet of air for 1~2 min, the pressure cooker was removed from the heat source, naturally cooled for 10~15 min, and water flushed and cooled to room temperature, slides were taken out and washed with water for 2 min, and with 0.01M pH=7.4 PBS for 2 min \times 3 times, and add a drop of 0.01M pH=7.4 PBS on the slides;
- (4) Drops of 3% hydrogen peroxide were added on the sections to inhibit endogenous peroxidase, incubated at room temperature for 10 min, rinsed with PBS for 3 min \times 3 times;
- (5) Draw a circle with an IHC oil pen at 3 mm from the tissue to prevent the loss of the titrated primary antibody and stabilize the antibody concentration;
- (6) Dropwise add the prepared primary antibody working solution, incubate in the wet box at 37 °C for 1 hour, rinse with PBS for 3 min \times 3 times;
- (7) Remove the PBS solution, add 1 drop or 50 μ L of polymer enhancer (reagent A) to

each tissue section and incubate in the chamber for 20 min, PBS rinse for 3 min×3 times;

(8) Remove the PBS solution, add 1 drop or 50 μ L of enzyme-labeled anti-mouse/rabbit polymer (reagent B) on each tissue microarray and incubate at room temperature for 30 min, PBS rinse for 3 min ×3 times;

(9) Add drops of freshly configured DAB color development solution, control color development under the naked eye for 1 to 3 min, generally not more than 5 min, rinse with tap water for 2 min; then re-stain with modified alcohol-free Carazzi hematoxylin solution for 40 s, rinse with water for 1 min, return to blue with PBS for 15 s, rinse with running water for 5 min;

(10) DAB color film was dehydrated by gradient alcohol and baked, sealed with neutral gum and coverslip, and observed under microscope.

3. Masson staining

(1) Sections recommended thickness 3-8 μ m, paraffin sections routinely deparaffinized to distilled water.

(2) 1:1 mix reagents A1 and A2 to prepare Weigert iron hematoxylin staining solution, plus cover the section staining for 5-10 min.

(3) Wash away excess staining solution with distilled water, add drops of acidic ethanol differentiation solution to differentiate for 5-15 s, and wash with distilled water for 30 s.

(4) Masson blueing solution to return to the blue 3-5 min, distilled water wash 30 s.

(5) Lichun red magenta staining solution staining 5-10 min.

(6) During the above operation, configure the weak acid working solution according to the ratio of distilled water: weak acid solution=2:1, and dropwise wash the weak acid working solution for 30 s, and pour off the excess liquid, and dropwise wash for 30 s.

(7) Pour off the excess liquid, add phosphomolybdic acid solution for 1-2 min, and wash with weak acid solution for 30 s. and pour off the excess liquid, add phosphomolybdic acid solution for 30 s.

(8) Pour off excess liquid, add aniline blue staining solution dropwise for 1-2 min. add weak acid working solution dropwise for 30 s.

(9) 95% ethanol rapid dehydration 2-3 s, anhydrous ethanol dehydration 2 times, each time 5-10 s.

(10) Xylene transparent 2 times, each time 1-2 min, neutral tree glue sealing.

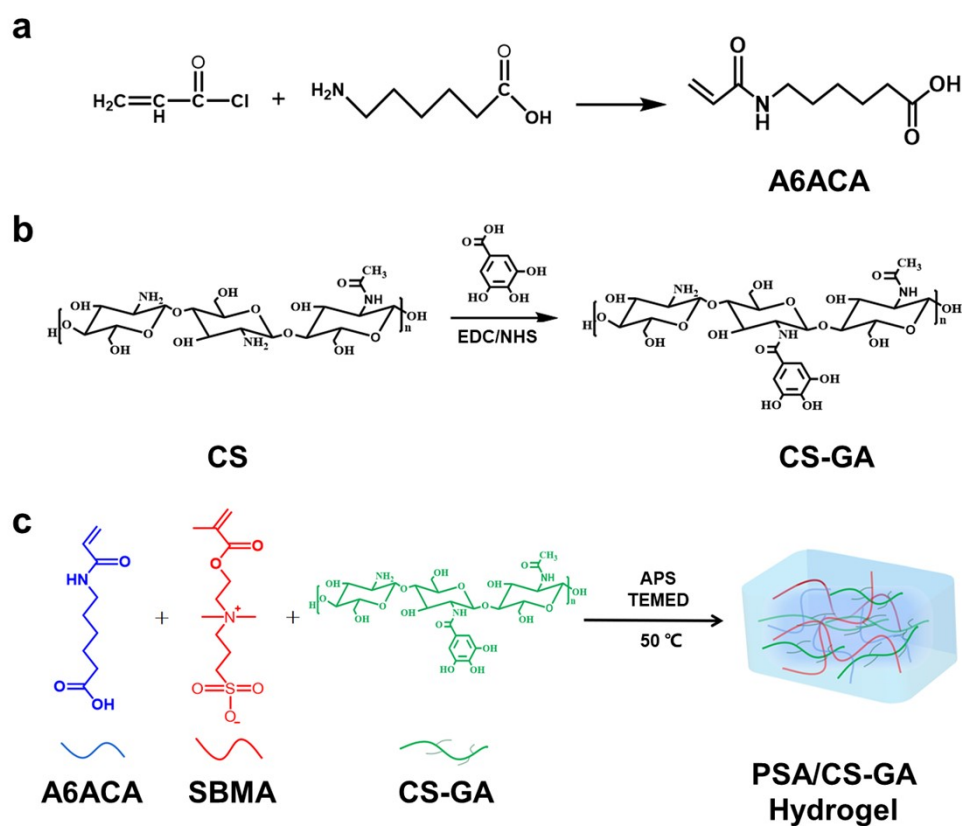


Fig. S1 Synthesis process of (a) A6ACA, (b) CS-GA, and (c) PSA/CS-GA hydrogels.

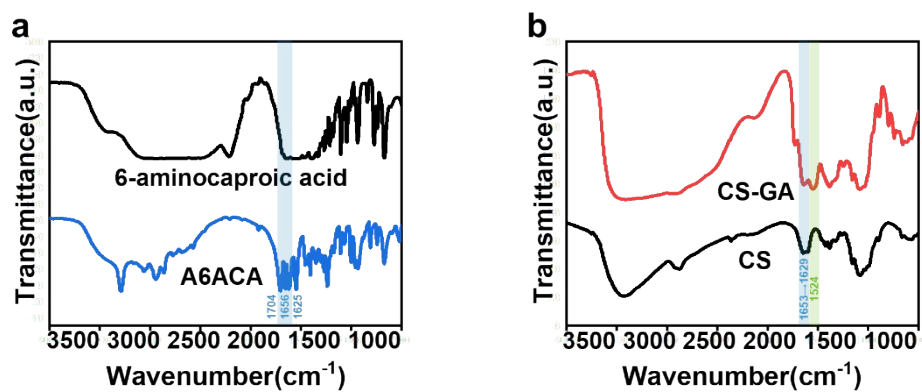


Fig. S2 (a) FT-IR spectra of A6ACA. (b) FT-IR spectra of CS and CS-GA.

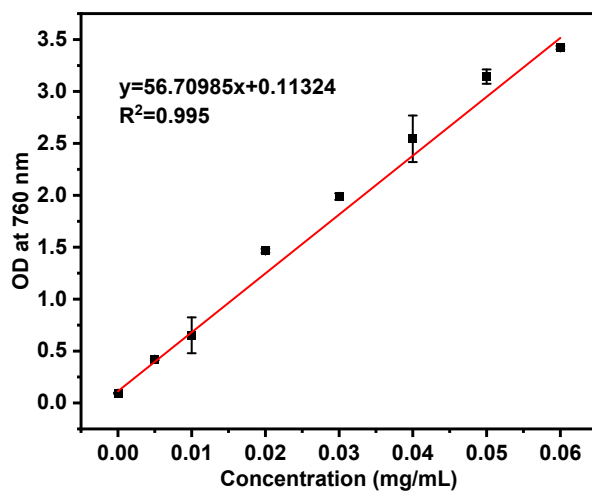


Fig. S3 Concentration standard curve of GA.

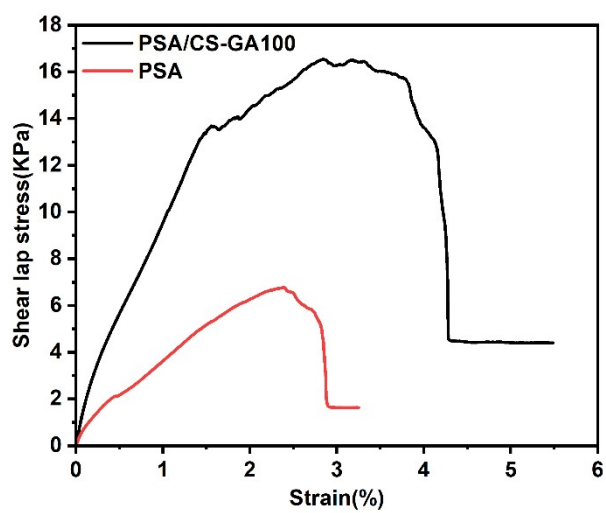


Fig. S4 Adhesion properties of PSA and PSA/CS-GA100.

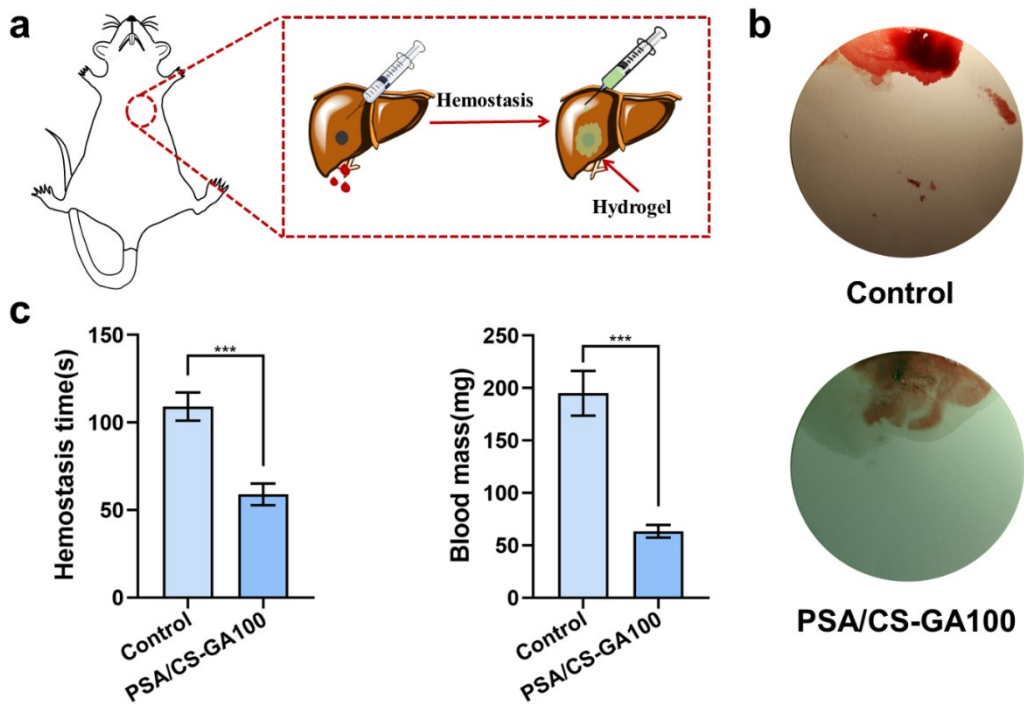


Fig. S5 PSA/CS-GA hydrogel hemostatic performance test. (a) Schematic representation of rat liver hemostatic model. (b) Pictures of bleeding volume on filter paper in the control and PSA/CS-GA hydrogel. (c) Hemostasis time and bleeding mass of PSA/CS-GA100 hydrogel. *** $p < 0.001$.

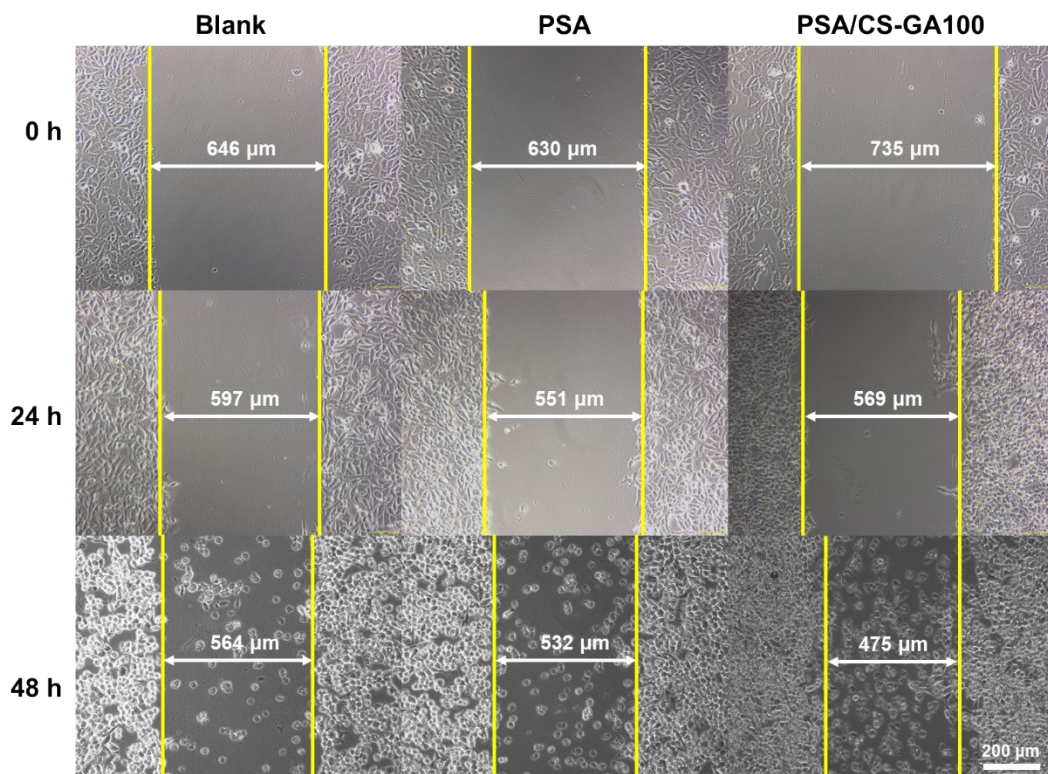


Fig. S6 The results of cell scratch healing.

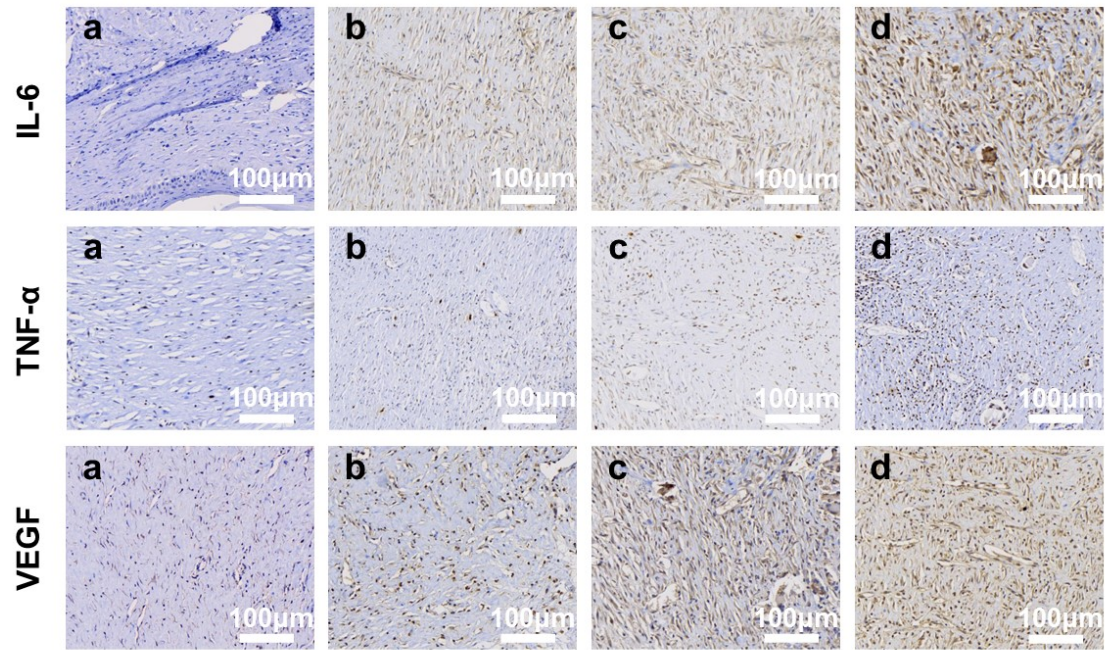


Fig. S7 Immunohistochemical staining. The staining intensity is (a) negative, (b) weak, (c) medium, and (d) strong, respectively.