Supporting information

Bioactive electrospun polylactic acid/chlorogenic acid modified chitosan bilayer sponge for acute infection wound healing and rapid coagulation

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1 Methods

1.1 Surface morphology

The surface morphology characteristics of 2D&3D CCT sponge and PLA nanofiber membranes were evaluated by scanning electron microscopy (SEM). Before observation, the surface of the sponge was sprayed with coarse gold, while the surface of the nanofibers was sprayed with fine gold.

1.2 Characterization of CS-CGA

Fourier transform infrared (FTIR) spectrometer was used to characterize the chemical structure of CS-CGA and verify that CGA was successfully grafted onto CS. Spectroscopy was performed on the band between 450 and 4000 cm^{-1}. Then ^{1}H NMR were performed to verify the successful grafting of CGA molecules onto CS backbones. The samples were dissolved in D_2O solution (1%) for NMR analysis with a 500 MHz NMR spectrometer.

1.3 Scanning electron microscope (SEM)

The morphology of PLA film was observed by SEM after its surface was sprayed with fine gold particles. A thin slice in cross sections of the CCT sponge was separate using a pair of tweezers, and the sliced surface was sprayed with coarse gold particles before observation.

1.4 Cytotoxicity

Cytotoxicity was assessed by using the tetramethylazolium blue (MTT) assay. Human umbilical vein endothelial cells (HUVECs) and NIH/3T3 cells were cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin. Cells were seeded on a 96-well plate at a density of 1×10^4/well. At the same time, 100 mg of CS-CGA, CCT and PCCT were placed in 1 mL of culture medium and extracted for 24 h. The cells were cultured in an incubator at 37°C, 5% CO_2 and 95% humidity. After 24 hours, the upper layer of

the culture medium was removed, 100 μL of sponge extract was added to each well and cultured for 48 hours. MTT was then added to each well and incubated for 4 h, then the supernatant was discarded, and 100 μL DMSO was added to each well. After shaking the 96-well plate on a shaker for 5 minutes, a microplate reader was used to measure the absorbance of the sample at 490 nm. Cell viability was calculated using formula (2-3).

$$Cell\ viability\ (\%) = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100 \quad (2\text{-}3)$$

All experiments were repeated three times.

1.5 TA release behavior

Sponges of equal mass, including CCT and PCCT, were placed in a centrifugal tube with 5 mL PBS and then transferred to a constant temperature shaking table at 37℃ for incubation at 100 rpm. Samples were taken at 0, 10 min, 30 min, 1, 2, 6, 12, 24, and 48 h intervals; each time point involved removing 200 μL of the extract and adding an equivalent volume of fresh PBS. The concentration of TA was determined using ninhydrin colorimetry. Upon reaction of TA's amino group with ninhydrin followed by heating above 90℃, a blue-purple Luhrmann violet product was formed with maximum absorption at 565 nm. The absorbance of the solution at 565 nm after the reaction was measured using a microreader. By comparing this measurement with a standard curve, the concentration of TA in the centrifuge tube at each time point was obtained to calculate the cumulative release of the drug.

1.6 Hemolysis behavior

Fresh whole blood from SD rats were obtained in a heparin sodium permeable centrifuge tube containing PBS and centrifuged at 1000 rpm for 5 min. Plasma and coagulated blood were removed, the remaining part was washed with PBS (pH 7.4) three times. The supernatant was removed, and the red blood cell (RBC) were diluted approximately 50 times. Sponges extract was incubated with diluted RBC suspension

at 37°C for 2 h. The positive control was distilled water, and the negative control was PBS buffer. After incubation, the mixture was centrifuged at 1000 rpm for 5 min to collect the supernatant which was added to a 96-well plate. The absorbance at 436 nm was measured. The hemolysis rate of each group was calculated through formula (2-4).

$$Hemolysis\ rate\ (\%) = \frac{OD_s - OD_{(-)}}{OD_{(+)} - OD_{(-)}} \times 100 \quad (2\text{-}4)$$

Among them, OD_s is the absorbance of the sample, $OD_{(+)}$ and $OD_{(-)}$ are the absorbance of the positive control and negative control, respectively.

1.7 Cell migration

NIH/3T3 and HUVECs were seeded into a 6-well plate at a density of 2×10^6/well and cultured in an incubator at 37°C with 5% CO_2 until the cells grew to cover the entire plane. A 10 μL-shaped tip was used to scrape a straight line on the cell surface. The free cells were washed twice with PBS buffer. Sponge extract that extracted with complete medium were added 1 mL to each well. The scratched area was observed and photographed at 0, 12, and 24 h, and each experiment was repeated 5 times.

1.8 Immunohistochemistry and fluorescence analysis

For histomorphology analysis, all rats were sacrificed on the 14th day, and the internal organs (heart, liver, spleen, lung, kidney) and wound tissues of the rats were removed and fixed with 4% paraformaldehyde and embedded with paraffin. All wound tissue site sections were stained with hematoxylin and eosin (H&E), Masson, and TNF-α to assess wound repair effectiveness. Then, wound tissue sections were incubated with PCNA antibody for 8 h at 4°C. Sections were then washed three times with PBS and incubated with secondary antibodies for 4 h at 4°C in the dark. The tissue was then observed using an inverted fluorescence microscope. Quantitative analysis was performed using ImageJ software.

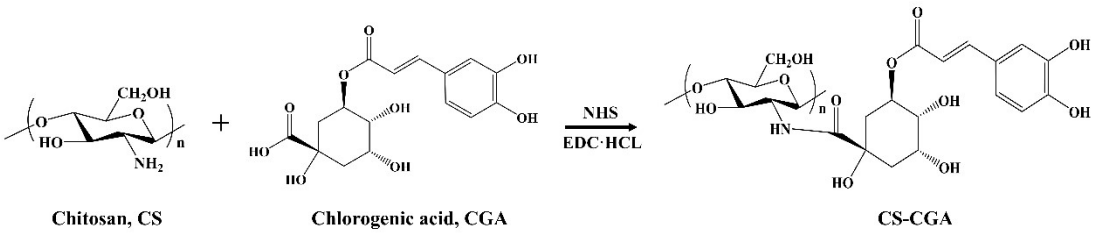

Figure S1. CS-CGA synthesis approach.

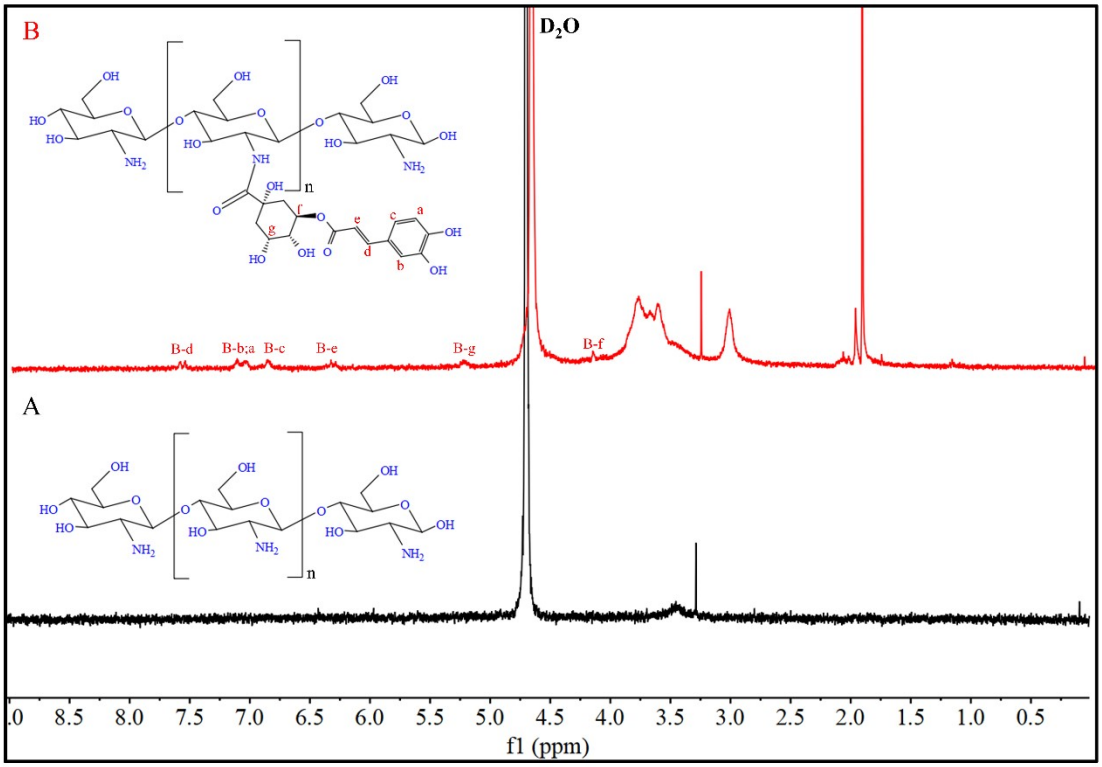

Figure S2. ^{1}H NMR spectra of (A) Chitosan (CS); (B) Chitosan grafted with chlorogenic acid (CS-CGA).

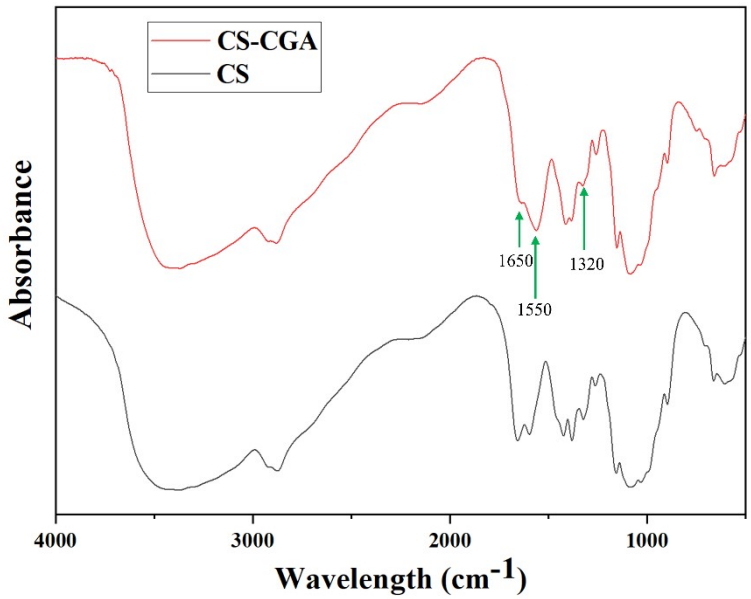

Figure S3. FTIR spectrum of CS and CS-CGA.

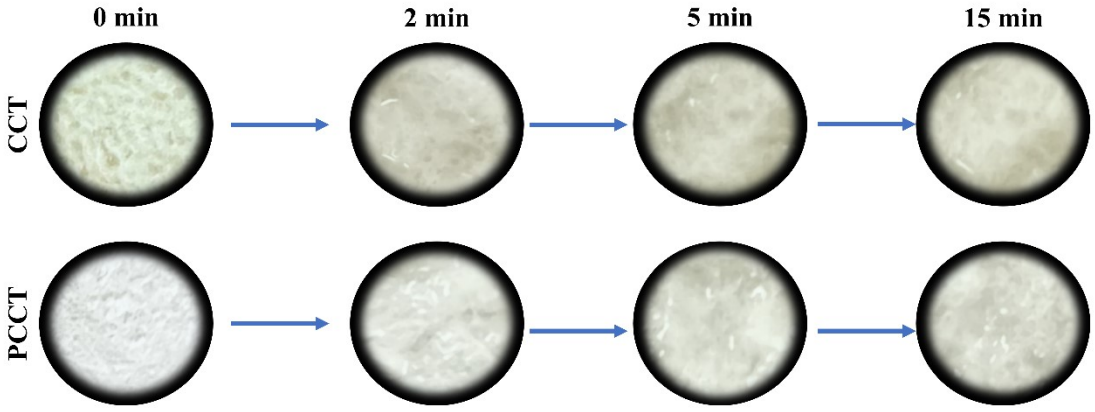

Figure S4. The dynamic process of CCT and PCCT sponge swelling kinetics.

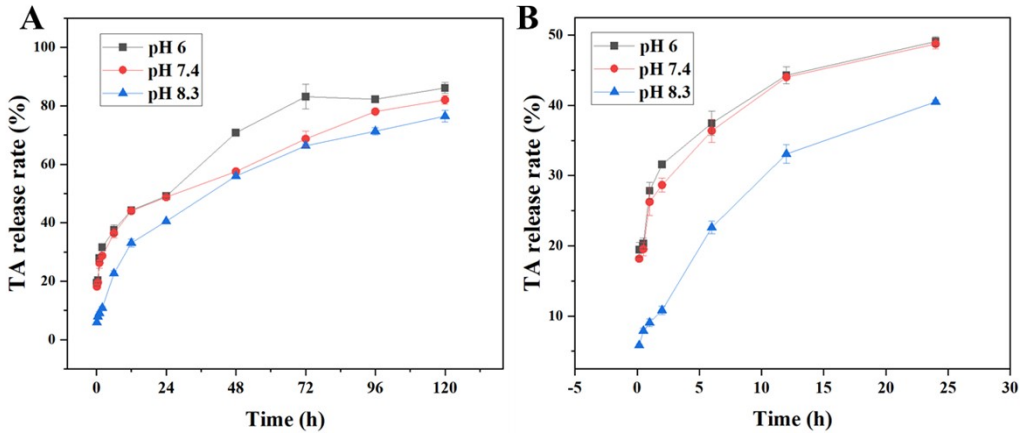

Figure S5. TA release curves at pH 6,7.4, and 8.3 for (A) 96 h and (B) 24 h.

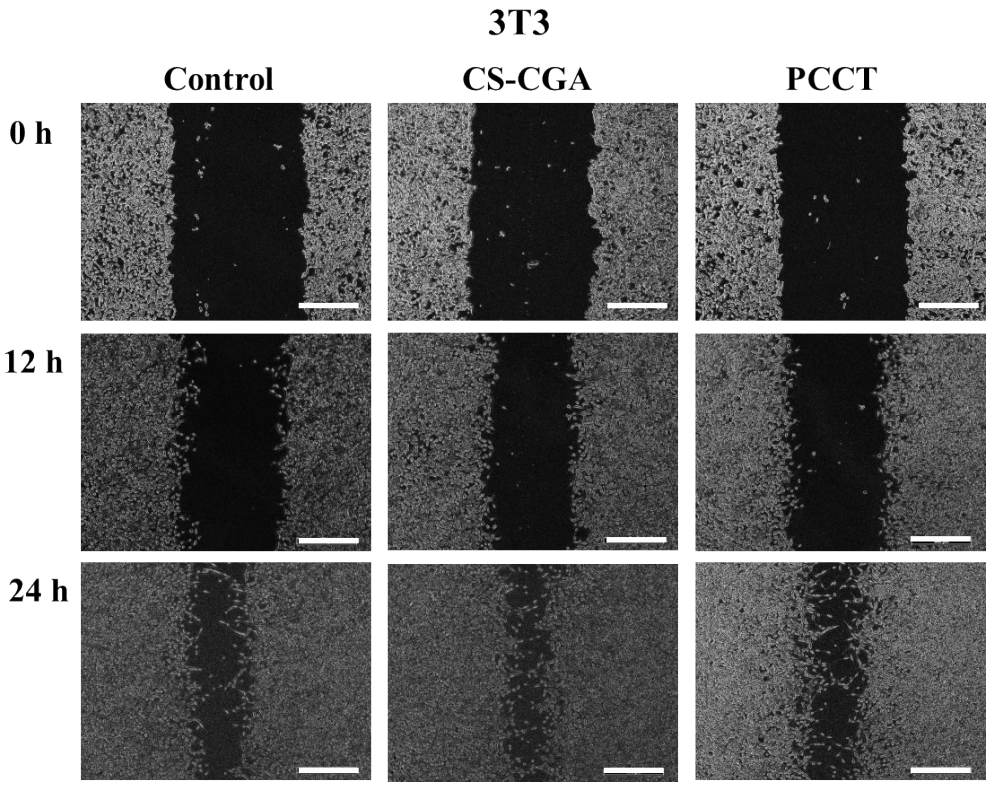

Figure S6. Scratch conditions on 3T3 under different treatments at 0, 12, and 24 h. The scale in the diagram represents 200 μm.

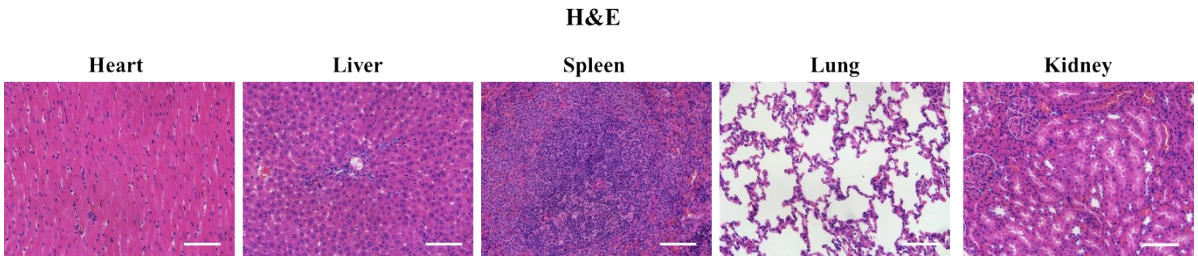

Figure S7. Visceral tissue stained by H&E of rats after treated with PCCT.

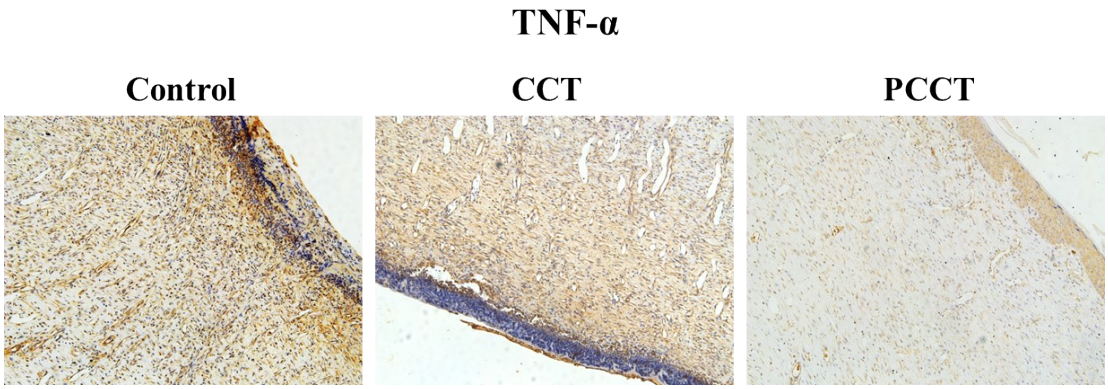

Figure S8. TNF-α immunohistochemical staining of wound tissue after treated with different methods.