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Supporting Information for

A Single-Component Photo-crosslinked Chitosan Hydrogel with Inherent Antimicrobial and Antioxidant Capabilities for Wound Healing

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Materials and methods

Materials

Chitosan (CS, *M*w = 200 kDa, deacetylation degree > 90%), lipoic acid (LA), and glycidyltrimethylammonium chloride (GTMAC) were purchased from Adamas Reagent, Ltd., China. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), and 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China).

Characterization

The ultraviolet-visible (UV-vis) spectra was obtained with an Agilent Gary 60 G6860A. The chemical structure of the LQCS was confirmed by nuclear magnetic resonance (^{1}H NMR, AV III HD 400 MHz, Bruker), and D_2O was used as deuterated solvents. The infrared spectra of LQCS were analyzed by flourier transform infrared (FTIR) spectroscopy (Bruker, Germany). The rheological properties of the hydrogel were determined using a rheometer (Anton Paar, MCR 302) equipped with a 20 mm diameter parallel plate and a 1 mm gap between the parallel plate and the measuring platform. Subsequently, the hydrogels underwent a series of rheological tests. First, scanning tests were used to analyze the modulus (storage modulus, G' and loss modulus, G'') versus frequency (0.01 ~ 100 Hz). The linear viscoelastic region of the hydrogel was investigated, and a strain scan test was performed at a constant frequency (1 Hz) with an oscillatory strain from 0.1% to 1000%.

ABTS and DPPH scavenging ability

The antioxidant capacity of hydrogels was evaluated using DPPH and ABTS free radicals. For DPPH scavenging assay, fresh DPPH/ethanol solution (0.1 mM) was prepared.[32, 33] Then the samples were submerged in the DPPH solution and left in the dark at 37°C for 30 min. Finally, the absorbance of each sample at 517 nm was recorded. Radical scavenging activity was calculated as the following formula:

$$Scavenging\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_1 is the absorbance of the experimental group and A_0 is the absorbance of DPPH solution.

For ABTS scavenging assay, the $ABTS^+$ solution was first obtained by reacting 7 mM $ABTS^+$ stock solution (10 mL) with 2.45 mM potassium persulfate solution (10 mL) overnight in the dark. The $ABTS^+$ solution was diluted 100 times and used for subsequent measurements. Scavenging activity was assessed by measuring the change in absorbance at 734 nm after mixing the sample with $ABTS^+$ for 30 min. The next steps are the same as for the DPPH scavenging assay. All tests were performed three times. The free radical scavenging activity was calculated similarly to the DPPH assay.

Adhesion Tests

The adhesion strength of the hydrogel to the skin was investigated by a modified lap-shear test. Tissue adhesion of the hydrogel was determined using fresh pig skin and gelatin-coated slides. Briefly, pig skin was cut into rectangular sample strips of 20 mm × 50 mm. The gelatin-coated slides were finally obtained by dropping a 20% (w/v) gelatin solution on the slide surface at 37°C and then dried overnight at room temperature. Next, 100 μL of hydrogel precursor solution was placed between the pig skin and gelatin-coated slides, followed by UV cross-linking, where the overlap area was 15 mm × 20 mm. The shear strength of the specimens was tested using a servo control system general purpose testing machine (AI-7000S), with a tensile rate of (5 mm/min), and the shear strength of the bioadhesive was determined at the separation point (n = 3).

In vitro Antibacterial Activity Test

For antimicrobial activity testing, slightly modified from previous literature, *E. coli* and *S. aureus* were selected as representative Gram-negative and Gram-positive bacteria, respectively. The antimicrobial properties of the hydrogels were evaluated using the plate counting method. In particular, 200 μL of LQCS-3 hydrogels were prepared in 48-well plates (PBS was used as a control), and then 100 μL of bacterial

solution (PBS, 1 × 10^6 CFU/mL) was added to each well. The treated bacterial suspension was diluted 100-fold and 100 uL was taken and spread evenly on a Luria-Bertani agar medium, incubated at 37°C for 16 h and then photographed and counted, with three parallel replicates in each group).

Cytotoxicity Evaluation

The L929 cells were incubated with the hydrogel solution at 37 °C and 5% CO_2 for 24 h. A 96-well plate was prepared with 100 μL of L929 cell suspension (5 × 10^4 cells/mL) per well, and the cells were cultured for 3 days. The CCK-8 reagent (KG087, KeyGEN BioTECH, Nanjing, China) was added to the cells for 1 hour, and the optical density was measured at a wavelength of 450 nm using a microplate reader. Additionally, a cell live and death assay was performed using a buffer containing Calcein AM and propidium iodide (PI). After incubation with the buffer for 30 min, the fluorescence images were taken using fluorescence microscopy (Leica Dmi8, Germany) with excitation wavelengths of 488 nm and 543 nm. In the flow cytometry experiment, we gathered the cells and centrifuged the cell suspension sample for 5 min. Afterward, we removed the liquid above the cell pellet, rinsed the cells two times with PBS, and repeated the centrifugation step to obtain concentrated cell sedimentation. To prepare the cells for analysis, we resuspended them in 500 μL of binding buffer. We added 5 μL of Annexin V. Following that, we included 5 μL of PI and gently mixed the solution (KGA1030, KeyGEN BioTECH, Nanjing, China). The mixture was then incubated at room temperature, shielded from light, for 10 min. Finally, we evaluated and analyzed the samples using Cytoflex and CytExpert software (version 4.0, Beckman Coulter, Inc).

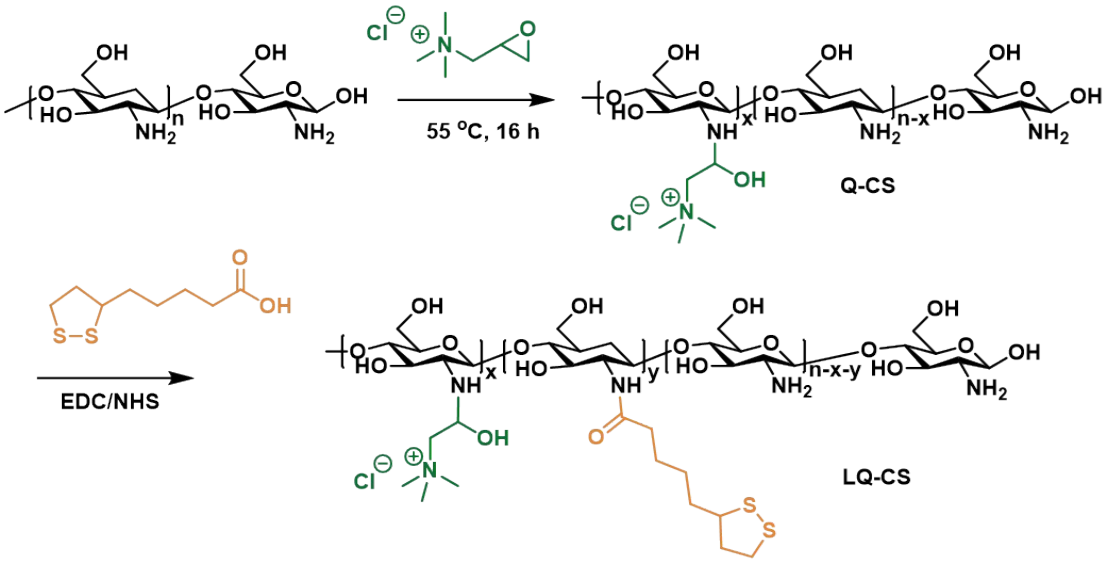

Figure S1. Synthesis route of the LQCS.

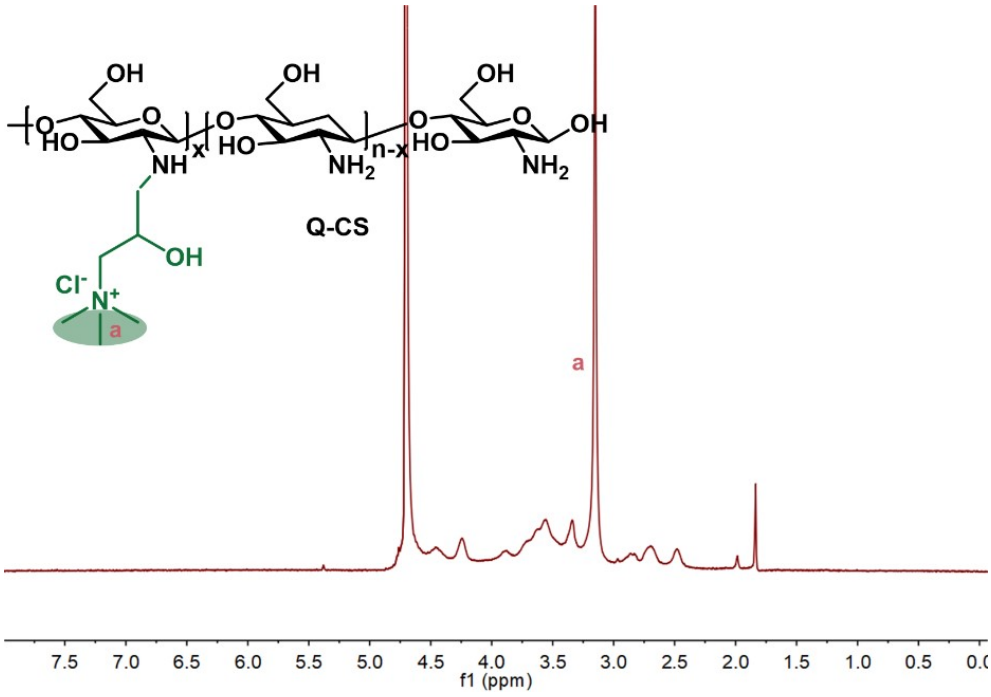

Figure S2. ^{1}H NMR spectra of QCS.

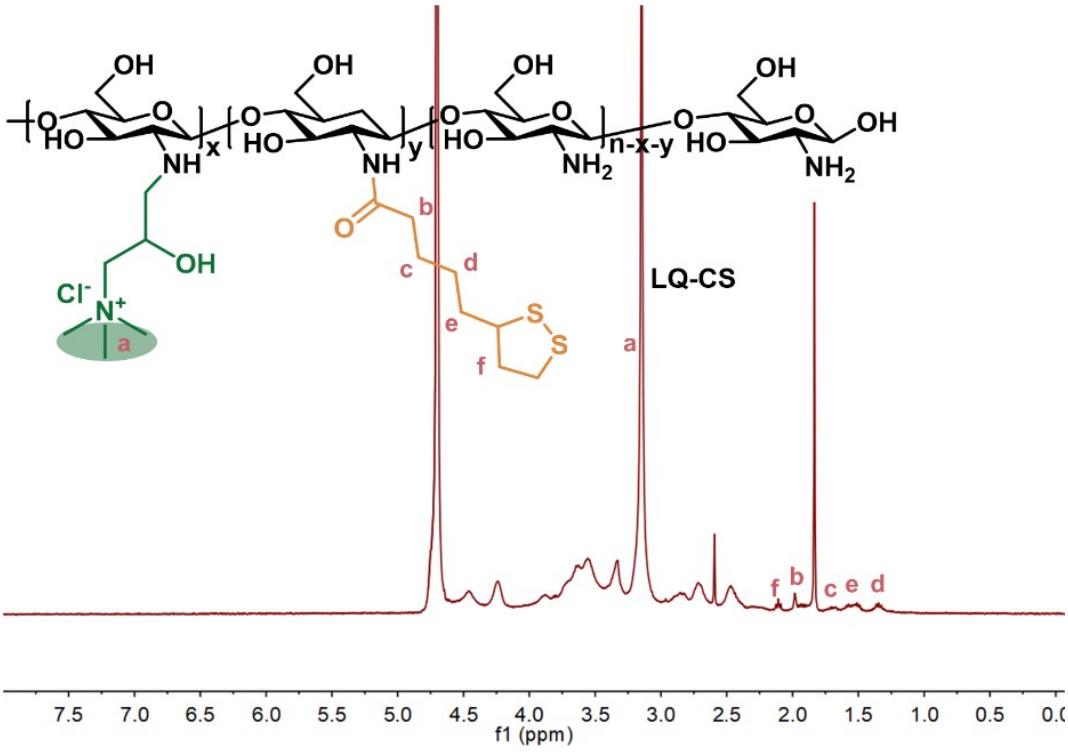

Figure S3. ^{1}H NMR spectra of LQCS.

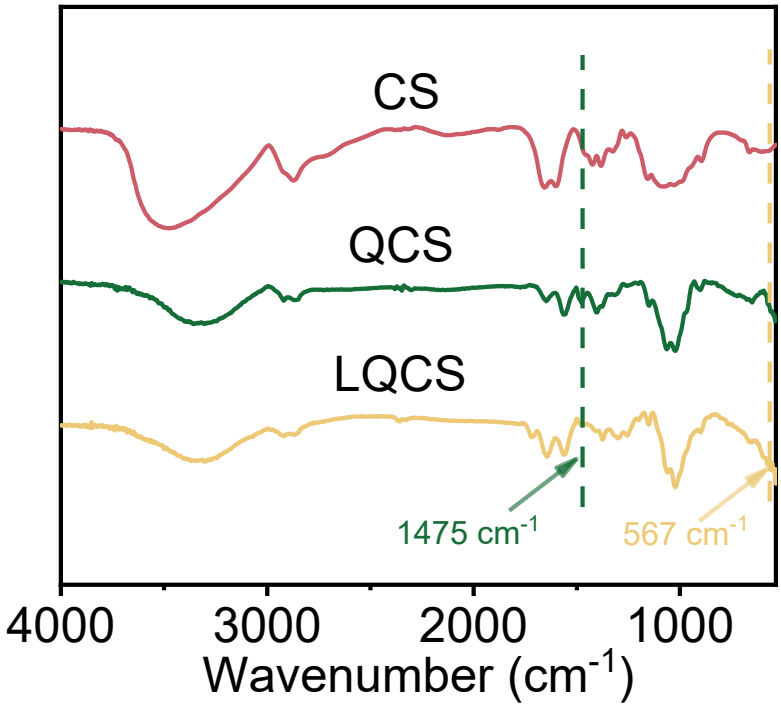

Figure S4. FTIR spectra of CS, QCS and LQCS.

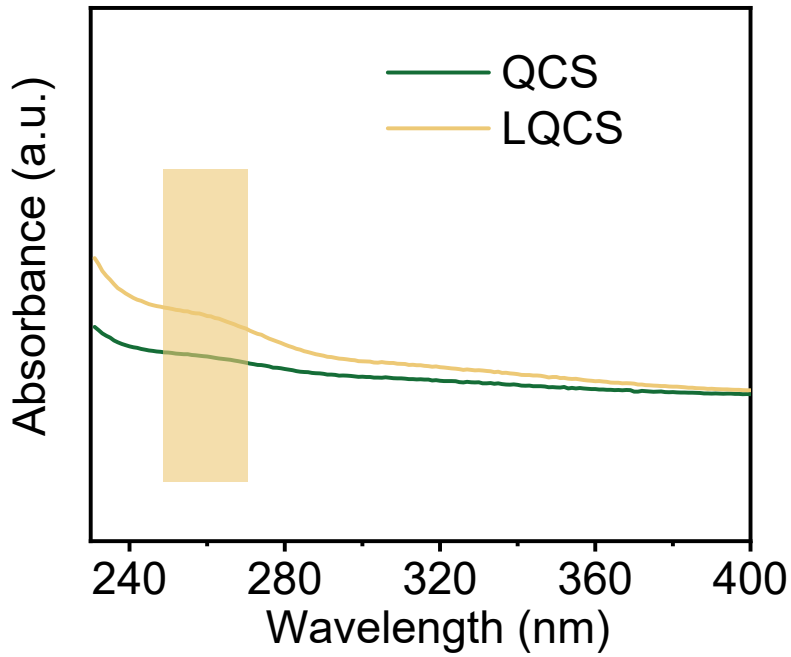

Figure S5. the UV-vis spectra of LQCS.

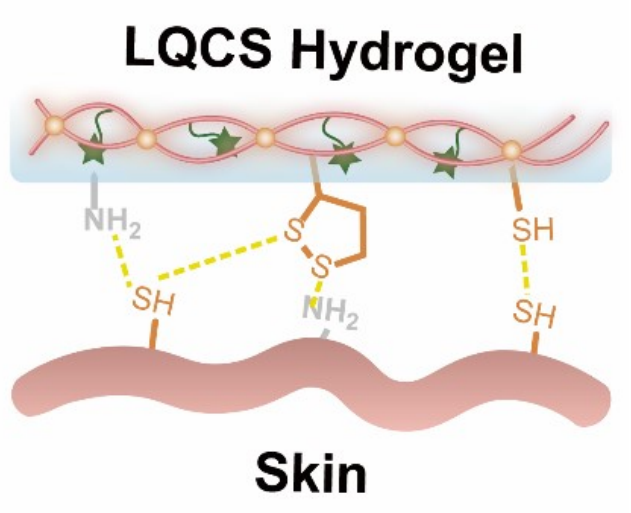

Figure S6. Adhesion mechanism of LQCS hydrogels.